CANCER RESEARCH

A MONTHLY JOURNAL OF ARTICLES AND ABSTRACTS REPORTING CANCER RESEARCH

VOLUME 2 AUGUST, 1942 NUMBER 8

Observations on the Mammary Tumor Incidence of Mice Born from Transferred Ova*

Elizabeth Fekete, M.A., and C. C. Little, Sc.D.

(From the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine)

(Received for publication April 27, 1942)

The successful transplantation of mammalian ova has been accomplished by several investigators. Heape (4, 5) transferred ova of an Angora doe rabbit, mated with an Angora buck, 32 hours after mating. The ova were in the 4 cell stage. The recipient was a Belgian hare doe which had been mated with a Belgian buck 3 hours before the transplantation. The ova were introduced into the upper end of the uterine tube. The recipient gave birth to 6 rabbits, 4 of which were Belgians and 2 Angoras. Hammond (3) was also successful in transferring rabbit's ova. Nicholas (9) transplanted ova which were removed from the uterine tubes of hooded rats into the uterus of white rats. Little, 1935, transferred ova of dba mice into C57 black mice and successfully raised 2 female and 1 male dba mice.

In the present experiment large numbers of ova were transferred from the high mammary tumor dba strain to the low mammary tumor C57 black strain and *vice versa*. The animals were divided into the following 4 groups:—

Group	Donor of ova	Recipient of ova	Nursed by
1	dba	C57 black	C57 black
2	C57 black	dba	dba
3	dba	C57 black	dba
4	C57 black	dba	C57 black

The animals belonging in the first 2 groups will be discussed in this article.

The general procedure, material, and method were as follows:—

Both the donors and the recipients of the ova were mated with their own brothers, and pregnancy was timed from the appearance of the vaginal plug. The ova were removed from the oviducts of the donor and were transferred into the uterus of the recipient. Trial ova transfers were performed at about 28, 52, and 76 hours after the observation of the vaginal plug. At 28 hours the ova are at the 2 to 4 cell stage and transplantation is seldom successful. At 52 hours the ova are at the 8 to 10 cell stage, are easily recovered from the oviduct, and implant and develop successfully in the uterus of the recipient. Transplantation is also successful at 76 hours, when the ova are at the morula or blastula stage, but because at this time they are situated at the lower end of the oviduct, where the lumen is narrow and the muscular wall thick, it is somewhat more difficult to recover them.

The male was placed in a pen with several females at about 4 p.m. and the females were examined for vaginal plugs the following morning at about 9 a.m. The female to be used as donor was killed 52 hours after a plug was observed. The uterus, oviducts, and ovaries were removed and placed in a watch glass containing a few drops of Locke's solution at about body temperature. The method of Lewis and Wright (6) was followed in securing the ova. They were collected by suction in a fine glass pipette and were discharged into a drop of Locke's solution placed in the concavity of a hanging drop slide. From here the desired number of ova was taken up and transferred with the same pipette.

The recipient female was anesthetized by intraperitoneal injection of nembutal. A small midventral incision, which was made about half an inch above the clitoris, permitted access to both horns of the uterus. Each uterine horn in turn was punctured with a hypodermic needle and through this puncture the glass pipette containing the desired number of ova was inserted pointing cephalad. The ova, with a small amount of the solution, were gently blown into the horn. Air accidentally introduced resulted in the failure of transplantation, and this had to be carefully avoided, therefore, by leaving some of the solution in the pipette. The contraction of the myometrium closed the puncture in the uterus, while the incisions of the

* This investigation was aided by grants from The Rockefeller Foundation and the National Cancer Institute.

abdominal wall and the skin were closed separately by a few silk sutures.

Trial transfers were performed, varying the length of pregnancy of the recipient. The following variations were tried:—

Variations in the length of pregnancy	
Of donor	Of recipient
hours	hours
52	28
52	52
52	76

Transplantation was most successful in the 2nd group. Here the donor's ova, transferred into the uterus of the recipient, had a slight advantage over the recipient's own ova, which did not enter from the oviduct into the uterus until about 20 hours later.

In several cases the oviduct leading to the uterine horn into which ova were transferred was cut to prevent the entrance of the recipient's own ova. Although in a few cases the transferred ova developed in the uterine horn of the cut side, in many cases this technic seemed to prevent the completion of pregnancy.

Later observations have proved that not all the animals in which vaginal plugs were found gave birth to litters. This might have been due to failure of ovulation, intrauterine death and absorption, or to abortion. The operative manipulation at transplantation might have increased the number of such cases. Among a random sample of 140 animals on which ova transfers were performed, 38 (27.14 per cent) failed to give birth to litters. In 51 (36.42 per cent) all the young born were those developed from the mother's own ova. In each of the remaining 51 mice (36.42 per cent) some of the transferred ova developed successfully to full term.

Usually the ova of one donor were transferred into the uterine horns of 2 recipients. Although in all cases several ova (an average of 4) were transferred to each recipient, the development of 3 or 4 was infrequent, and most often only 1 or 2 ova developed to full term. The mortality among the young mice was high and about one-third of them died before they reached weaning age.

The total number of ova recovered from a random sample of 50 dba females was 434, with an average of 8.68 ova per animal, while in 50 C57 black females 390 (an average of 7.8 ova) were found. It seems interesting to compare these figures with the litter size of normal animals of each strain which had not been subjected to operation.

1. Total number of ova recovered in 50 dba ..... 434 Average 8.68
2. Total number of young born following normal pregnancy in 104 dba litters ..... 555 Average 5.33
3. Total number of ova recovered in 50 C57 black following normal pregnancy ..... 390 Average 7.80
4. Total number of young born following normal pregnancy in 192 C57 black litters ..... 742 Average 7.27

Evidently the intrauterine mortality in the dilute brown mice is higher than in the C57 black mice.

In most instances the recipient mice were allowed to give birth to the young, but in some the pregnant female was killed on the last day of gestation and the living fetuses were removed from the uterus. In either case the possibility that the young mice would get some milk of their own strain did not exist. The newborn C57 black mice have more pigment in their retinas than the dilute brown mice and are easily distinguishable from them at birth. The date of birth was very carefully noted in all cases. None of the females were nursing while pregnant. Table I shows the length of time from observation of the vaginal plug until birth.

TABLE I: LENGTH OF PREGNANCY IN DAYS

	dba females		C57 black females	
Days	Total number	Per cent	Total number	Per cent
18	1	0.51	1	0.58
19	23	11.79	67	39.23
20	132	67.64	89	52.35
21	36	18.46	20	7.64
22	3	1.53	0	0.00

C57 BLACK MICE BORN FROM OVA TRANSFERRED INTO DBA FEMALES AND NURSED BY DBA MOTHERS

A total number of 79 mice were born in this group and 47 were raised beyond weaning age (31 males and 16 females). Data have been completed so far on 23 males and 14 females. Some of the males were used for breeding. Two males developed skin infections and in 2 lymphoblastoma occurred. Most of them were killed showing signs of feebleness due to old age.

Among the 14 females one was kept virgin and lived 706 days without developing mammary gland tumors. Thirteen females were mated with transferred-ova males. Whenever possible, brother-to-sister matings were made and breeding was continued through several generations. Some of the lines are being continued at the present time. No offspring resulted from the mating of 3 females to 1 male (Nos. 55, 57, 59 × ♂56), presumably because of the sterility of the male.

Among the 10 breeding females, 5 (50 per cent) developed carcinoma of the mammary gland at an average age of 474 days, while the remaining 5 died without such tumors at an average age of 442 days.

The first and all further generations were produced by brother-to-sister matings. From the first generation on the animals nursed their own C57 black mothers. Mothers were not allowed to nurse all their litters because of lack of space. They were "force bred" during part of their breeding period. Force breeding increases the number of litters. To ascertain whether this had any effect on the incidence of mammary carcinoma the females were divided into 2 groups: those which had 1 to 6 litters and those which had 7 to 13. The percentage of mammary growths in each group was calculated and shows that no significant difference existed. The data are presented in Table II.

Previous investigations (7, 2) also showed that force breeding did not increase the mammary tumor incidence of C57 black and dba mice.

TABLE II: RELATION OF MAMMARY TUMOR PERCENTAGE TO NUMBER OF LITTERS

	With tumors	Without tumors	Total	Incidence of tumor, per cent	Average age, days
Females with 1 to 6 litters	37	13	50	74	437
Females with 7 to 13 litters	31	12	43	72.1	443

Among the descendants of the 5 breeding females which died without tumors of the mammary glands such growths occurred in the 1st generation. This indicates that all 10 transferred-ova animals differed from C57 black animals by transmitting a tendency to form carcinoma of the mammary gland in their offspring.

First generations were raised and data completed of 8 matings. Thirty-five females lived beyond 210 days and 22 developed neoplasms of the mammary glands (62.85 per cent) at an average age of 484 days. The remaining 13 died without mammary tumors (average age 487 days).

Thirty-four females were raised in the 2nd generation. Twenty-eight of them (82.35 per cent) developed mammary carcinomas at an average age of 464 days, while 6 died without them (average age 368 days).

In the 3rd generation 24 females were raised. Mammary neoplasms occurred in 18 of them (75 per cent) at an average age of 397 days, while 5 died without such tumors (average 410 days).

The length of life, autopsy records of the original transferred-ova females, and an analysis of the individual matings and subsequent generations are presented in Table III.

Microscopic diagnosis of the mammary tumors

TABLE III: TUMOR INCIDENCE IN C57 BLACK FEMALES OBTAINED FROM TRANSFERRED OVA AND IN THEIR DESCENDANTS

Ledger number of females	Length of life, days	Autopsy records	Mated to male No.	Generations * First: Total number of females	First: Number of females with mammary tumors	Second: Total number of females	Second: Number of females with mammary tumors	Third: Total number of females	Third: Number of females with mammary tumors
28	569	Adenocarcinoma of mammary gland	7	5	4	10	9	9	8
126	416	Adenocarcinoma of mammary gland	124	3	1	4	4	2	2
22	440	Adenocarcinoma of mammary gland	22	3	2	..	..	..	..
5	579	Adenocarcinoma of mammary gland	4	..	..	..	..	..	..
21	366	Adenocarcinoma of mammary gland	22	..	..	..	..	..	..
86	311	No pathologic changes	87	3	3	8	7	4	2
3	580	Hygroma of cervical lymph node	2	7	4	8	5	7	4
20	570	No pathologic changes	22	6	3	4	3	2	2
24	509	Skin infection, subcutaneous edema	22	4	4	..	..	..	..
6	244	Paratyphoid	4	4	1	..	..	..	..
55	273	No pathologic changes	56						
57	809	Hemangioma of liver	56	No offspring					
59	293	Skin infection	56						
222	706	No pathologic changes	Not mated						
				35	22	34	28	24	18

Total number of mice 93
Number of mice with mammary tumor 68
Percentage of mice with mammary tumor 73

* Only those females are included which lived 210 days and had at least one litter.

proved them to be adenocarcinomas. Although variations existed, in most of them the stroma was dense and cellular, while the parenchyma contained numerous cysts and areas of hemorrhage were frequent.

Among the 37 females, 6 died young, while the remaining 31 lived an average of 493 days. None of them had tumors of the mammary glands. The females were mated with transferred-ova males and

TABLE IV: TUMOR INCIDENCE IN DBA FEMALES OBTAINED FROM TRANSFERRED OVA AND IN THEIR DESCENDANTS

				Generations *					
				First		Second		Third	
Ledger number of females	Length of life, days	Autopsy records	Mated to male No.	Total number of females	Number of females with mammary tumors	Total number of females	Number of females with mammary tumors	Total number of females	Number of females with mammary tumors
11	566	Paratyphoid	8	11	3	12	2	12	4
3	281	Paratyphoid	1	8	2	11	4	4	0
10	503	Paratyphoid	5	10	2	5	0	6	0
130	517	Diarrhea	129	5	1	2	0	..	..
134	630	Peritonitis	127	3	2	..	..	..	..
263	457	Diarrhea	262	3	1	..	..	..	..
261	250	Died during parturition	260	2	0	8	1	3	2
9	430	Paratyphoid	4	8	0	21	2	19	1
17	437	Paratyphoid	12	3	0	6	1	..	..
409	605	Lymphoblastoma	408	4	0	3	0	4	3
6	420	Paratyphoid	4	6	0	11	0	13	3
156	658	Lymphoblastoma	154	4	0	3	0	6	1
2	457	Paratyphoid	1	1	0	2	0	7	0
135	313	No pathologic changes	136	3	0	8	0	4	0
157	759	Lymphoblastoma	154	2	0	3	0	3	0
410	745	Ovarian cysts	408	6	0	7	0	6	0
16	437	Paratyphoid	12	1	0	1	0	..	..
60	504	Paratyphoid	34	1	0	4	0	..	..
74	275	Paratyphoid	34	1	0	2	0	..	..
155	343	No pathologic changes	154	1	0	2	0	..	..
258	407	Pneumonia	257	2	0	1	0	..	..
641	821	Feebleness, old age	640	3	0	3	0	Alive	
342	514	Pneumonia	285	5	0	..	..	..	..
391	771	Adenoma of ovary	199	2	0	..	..	..	..
412	484	Pneumonia	411	2	0	..	..	..	..
15	216	Accidentally killed	24	All died young					
315	628	Accidentally killed	262	All died young					
132	527	Diarrhea	219	All died young					
392	567	Cystic liver	199	All died young					
641	831	Ovarian cysts	640	Alive					
856	637	Hygroma of cervical lymph node	841	Alive					
				—	—	—	—	—	—
				97	11	115	10	87	14

Total number of mice 299
Number of mice with mammary tumor... 35
Percentage of mice with mammary tumor . 11.7

* Only those females are included which lived 210 days and had at least one litter.

DBA MICE BORN FROM OVA TRANSFERRED INTO C57 BLACK FEMALES AND NURSED BY C57 BLACK MOTHERS

One hundred and nineteen mice were born and 78 were raised beyond weaning age in this group (36 males, 42 females). Data have been completed so far on 34 male and 37 female mice. Several of the males died of paratyphoid, 2 had lymphoblastomas, 2 had nephritis, and 1 had a testicular tumor. Most of them were killed showing signs of feebleness and old age. here, too, whenever it was possible, brother-to-sister matings were made.

First generations were raised from 25 matings, and among the offspring of 6 matings, 11 females developed mammary growths (average age 465 days). A total number of 97 first generation females lived over 210 days and the incidence of mammary gland tumors was 11.34 per cent.

One hundred and fifteen 2nd generation females living more than 210 days were raised from 20 lines.

Mammary tumors occurred in 10 females (8.69 per cent, average age 469 days). Three lines in which these neoplasms did not occur in the 1st generation had tumorous offspring in the 2nd.

Twelve lines were continued and 87 females were raised in the 3rd generation. Mammary tumors occurred in 14 females (16 per cent, average age 506 days). Three lines in which they did not occur in the 2 previous generations had offspring with such tumors in this generation.

The length of life, autopsy records of the original transferred-ova females, and an analysis of the individual matings and subsequent generations are presented in Table IV.

All the mammary tumors were sectioned, examined microscopically, and diagnosed as carcinomas. In general the stroma of these tumors was loose while the parenchyma was rather dense and cellular.

TABLE V: COMPARISON OF FOSTER NURSING DATA OF VARIOUS SOURCES WITH THE PRESENT DATA

Type of mice	Source	Number of mice	Mammary tumor, per cent	Genetic influence	Uterine influence	Milk influence
C57 black fostered by dba	van Gulik and Korteweg (10)	67	13	—	—	+
	Murray (8)	98	9.2	—	—	+
C57 black ova transferred to dba nursed by dba	Present data	10	50	—	+	+
First and later generations	Present data	93	73	—	—	+
dba fostered by C57 black	van Gulik and Korteweg (10)	110	9	+	+	—
	Murray (8)	108	23.1	+	+	—
dba ova transferred to C57 black nursed by black	Present data	29	0	+	—	—
First and later generations	Present data	299	11.7	+	+	—

DISCUSSION

The fact that nursing newborn mice of a low mammary tumor strain on foster mothers of a high mammary tumor strain increases the mammary tumor incidence, while foster nursing in the opposite way decreases it, was established by Bittner (1).

van Gulik and Korteweg (10), as well as Murray (8), investigated the influence of foster nursing on the mammary tumor incidence of the same 2 strains of mice used in the present experiment. The former investigators reduced the incidence in dba virgin females from 85 to 9 per cent by fostering them on C57 blacks. In Murray's experiment the reduction was from 61.6 to 23.1 per cent. Foster nursing C57 black animals on dba, van Gulik and Korteweg increased the tumor incidence of virgin females from 0 to 9 per cent, and Murray from 0 to 9.2 per cent.

The mice used in the present experiment also can be considered as fostered, but in addition they were subjected to the intrauterine environment of their foster mothers during embryonic development. The genetic influence of all 3 experiments can be regarded as similar.

With the above statements in mind a comparison of the results seems interesting and is presented in Table V.

The comparison shows that in our experiment the mammary tumor incidence in the C57 black females has been increased (13 and 9.2 per cent to 50 per cent) while in the dba females it has been decreased (9 and 23.1 per cent to 0 per cent). The explanation that both the increase and decrease were due to the intrauterine influence is possible. However, other points should also be taken into consideration.

In the case of the C57 black females the fact that the data of van Gulik and Korteweg (10) as well as of Murray (8) were based on the observations of virgin females, while our experimental animals were breeding females, might account for some increase. The incidence of mammary tumors is usually higher in breeding females than in virgins.

In the dba females a decreased incidence of mammary tumors existed in spite of the fact that our experimental females were breeding, while the former investigators observed virgin females. It is possible that the animals of the former investigators may have had an opportunity to nurse their own dba mothers before being fostered. Such an opportunity did not exist in our experiment. That in spite of the complete absence of the milk influence mammary tumors occurred in the 1st and later generations is important and emphasizes the possibility of an intrauterine influence.

SUMMARY

Fertilized ova of the C57 black strain of mice were transferred, 52 hours after observation of a vaginal plug, into the uterus of dba mice and *vice versa*. The

mice born from transferred ova were nursed by mothers which gave birth to them. In addition to the foster nursing influence, these mice were subjected to the intrauterine environment of their foster mothers.

In the transferred-ova C57 black mice carcinomas of the mammary glands occurred in 50 per cent of the breeding females, while none of the transferred-ova dba animals had any mammary neoplasms. This increase and decrease in mammary tumor incidence is greater than has been accomplished by foster nursing alone in these 2 strains.

It is possible that the intrauterine influence is at least partially responsible for the changes in both increasing and decreasing directions.

In the descendants of the original transferred-ova C57 black mice (1st, 2nd, and 3rd generations) the mammary tumor incidence was 73 per cent.

The descendants of the original transferred-ova dba mice did not remain free from mammary growths. In the 1st, 2nd, and 3rd generations the incidence was 11.7 per cent.

At the present time neither group of descendants can be considered as significantly different from its respective group of original transferred-ova animals.

The technical assistance of Mrs. Allen Savage is gratefully acknowledged.—Authors.

REFERENCES

1. Bittner, J. J. Relation of Nursing to the Extra-Chromosomal Theory of Breast Cancer in Mice. Am. J. Cancer, **35**:90-97. 1939.
2. Fekete, E. Observations on Three Functional Tests in a High-Tumor and a Low-Tumor Strain of Mice. Am. J. Cancer, **38**:234-238. 1940.
3. Hammond, J. Verh. d. 1er Internat. Kanninchenzüchten Kongr. 1930.
4. Heape, W. Preliminary Note on the Transplantation and Growth of Mammalian Ova within a Uterine Foster Mother. Proc. Roy. Soc. London, s. B, **48**. 1890.
5. Heape, W. Further Note on the Transplantation and Growth of Mammalian Ova within a Uterine Foster Mother. Proc. Roy. Soc. London, s. B, **61**. 1897.
6. Lewis, W. H., and Wright, E. S. On the Early Development of the Mouse Egg. Carnegie Institution of Washington, Contributions to Embryology, No. 148:113-143. 1935.
7. Little, C. C., and Pearsons, J. The Results of a "Functional Test" in a Strain of Mice (C57 Black) with a Low Breast Tumor Incidence. Am. J. Cancer, **38**:224-233. 1940.
8. Murray, W. S. Studies on the Effect of Foster Nursing and Its Relation to the Development of Mammary Carcinoma in the Mouse. Cancer Research, **1**:790-792. 1941.
9. Nicholas, J. S. Development of Transplanted Rat Eggs. Proc. Soc. Exper. Biol. & Med., **30**:1111-1113. 1933.
10. van Gulik, P. J., and Korteweg, R. The Anatomy of the Mammary Gland in Mice with Regard to the Degree of Its Disposition for Cancer. Nederl. Akad. v. Wettenschappen, Proc. Sect. Sc., **43**:891-899. 1940.

The Origin of Some Inbred Mice*†

Leonell C. Strong, Ph.D.

(From the Department of Anatomy, Yale University School of Medicine, New Haven, Conn.)

(Received for publication March 30, 1942)

The introduction of the use of inbred mice into cancer and other biological research has been a long and slow process. Even at the present time the application of this technic is not by any means universal. A glance at Hartwell's recent extensive survey of compounds which have been tested for carcinogenic activity (13) will show that, although the use of inbred mice is largely appreciated, considerable work is still being done on mice of unknown or uncertain origin. For example, in this monograph there are approximately 3,747 references to the use of animals in cancer research. Sixteen groups of animals are mentioned. There are 2,202 references to the use of mice (58.7 per cent of grand total); 876 to rats; 339 to rabbits; 116 to guinea pigs; 95 to fowls; 34 to dogs; 32 to monkeys; and 53 references to the use of the other 9 minor groups of experimental animals. The 2,202 references to the use of mice can be separated into 4 classes as follows: (a) 883 to mice whose origin is not stated; (b) 532 (24.1 per cent of the total use of mice) to the inbred strains developed by the present author, the origin of which will be discussed in this paper; (c) 414 to the use of "heterozygous" [1] mice (many of the "heterozygous" mice used were obviously produced by hybridization from our inbred mice, but are not included, however, in the computed 24.1 per cent; and (d) 373 references to mice from all other sources. That is, when the source of mice is definitely stated (1,319 times) the use of the inbred strains of Strong was 40.3 per cent of the total use of mice (exclusive of derived heterozygous animals). This percentage would probably be even greater since many of the references to stocks are too vague for proper classification; *e.g.*, (a) black agouti, (b) inbred, (c) cancer-resistant, (d) cancer-susceptible, and (e) inbred albino.

It is the conviction of many geneticists that the use of the inbred mouse in cancer research has made possible many contributions of a fundamental nature that would not have been made otherwise. Perhaps it would not be out of place to make the suggestion that within the near future all research on mice should be carried out on inbred animals or on hybrid mice of known (genetically controlled) origin where the degree of biological variability has been carefully controlled. A major step in this direction has been taken by investigators at the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, which has made available to others many of the inbred strains developed by them and from other sources. Their excellent book on the biology of the laboratory mouse (19) should fulfill a long felt need and should do much in emphasizing the desirability of using controlled mice in cancer and other biological research.

The application of genetic principles to cancer research by the author goes back nearly twenty-five years. The problems that confronted him first at that time were: (a) How could cancer research be placed on a quantitative basis, and (b) how would it be possible to apply genetic principles to a problem where so many unknown variables apparently were operative? The appreciation of the need of this type of quantitative biological research was perhaps even less then than it is now. In fact, a great approach to placing biological research on a quantitative basis had already been made by geneticists. A noteworthy attempt to cover adequately the application of quantitative methods in biology has been made by investigators at The Biological Laboratory, Cold Spring Harbor, Long Island. They have already conducted 9 annual Symposia on Quantitative Biology, beginning in 1933 (9).

A preliminary survey of the field in 1918 disclosed the fact that no inbred pedigreed [2] mice fulfilled the requirements for quantitative or genetic research. Guinea pigs had been inbred by Dr. Wright in Washington; rats at the Wistar Institute by Dr. Helen Dean King. The nearest approach to an inbred stock of mice was the Bagg albino, where some brother-to-sister matings had been employed but where most of the stock had been carried on by pen matings—several females and males in one cage—which merely means that the stock was continued without introducing mice of a foreign source. In 1920, Bagg (2b) reported on the origin of the albino usually referred to as the Bagg albino. In this communication he referred to these mice as the white family in which 8 inbred generations had been recorded. Between 1918 and 1920 the author outcrossed many albino mice obtained from Bagg to mice showing the triple recessive characters: dilute, brown, and nonagouti. The F_1's obtained were black agouti, brown agouti, black nonagouti, and brown nonagouti, which indicated that these albino mice were not homozygous at that time even for the common discernible color-producing genes.

Only 3 unpedigreed mice of the now well-known dba or dbr strain were then alive, sent by Dr. E. E. Tyzzer of Boston to Dr. C. C. Little at Cold Spring Harbor, Long Island, following the depletion of the original pedigreed dilute brown stock by an epidemic of murine paratyphoid. It was necessary, therefore, to produce stocks of inbred mice as the first requisite for applying genetic principles to cancer research, thus placing it somewhat on a quantitative basis.

* This investigation has been aided by grants from The Jane Coffin Childs Memorial Fund for Medical Research and The Anna Fuller Fund.

† Read at the meeting of the American Association for Cancer Research, Boston, Massachusetts, March 31, 1942.

[1] The use of the term "heterozygous" as applied to market or hybrid mice (not necessarily inbred) is greatly to be deplored. Heterozygous in genetics has the specific meaning of denoting that condition where the genetic determiners of heredity (genes) are in the heterozygous form. Since apparently several distinctive classes of "heterozygous" individuals are being used, it would be better to use either the nonspecific term "hybrid" or the more specific genetic terms of "F_1" or "backcross mice," etc. The relative values and proper use of inbred and heterozygous or hybrid mice has been very adequately discussed by Russell (19).

[2] By the term "pedigreed" is meant that system of mating where all animals are given an individual identifying number early in life, and the complete vital records of each animal are individually entered in permanent form.

The origin of the A (21), C (22), C3H (23), CBA (24), JK (25), F (26), and NH (27) strains has been published in various periodicals. It is the purpose of this paper to bring together in one place these data, together with others that may lead to a better understanding of the origins and genetic relationships of these many inbred mice that are being used by investigators in various parts of the world. Among other advantages of this procedure would be: (a) avoidance of further confusion in the literature, and (b) aid to investigations of research where some degree of genetic relationship between individuals of the various stocks may have an influence on the results obtained.

Fig. 1 shows in condensed graphic form the origin of, and genetic relationships between, 11 of those pedigreed inbred strains which were developed between the years 1920 and 1927. Between 1920 and 1925, 4 unpedigreed pairs of mice from various sources were selected and started on a pedigreed inbreeding (primarily a brother-to-sister mating) regime, and thus were produced in the ensuing years the A, C, CBA, C3H, CHI, and C12I strains. Between 1925 and 1927, 12 additional unpedigreed pairs of mice were selected, primarily from the colony of Dr. W. E. Castle of Bussey Institution, Harvard University. It was impossible to continue all the original 16 selected strains, and only 5 (A, F, I, L, and N) are still in

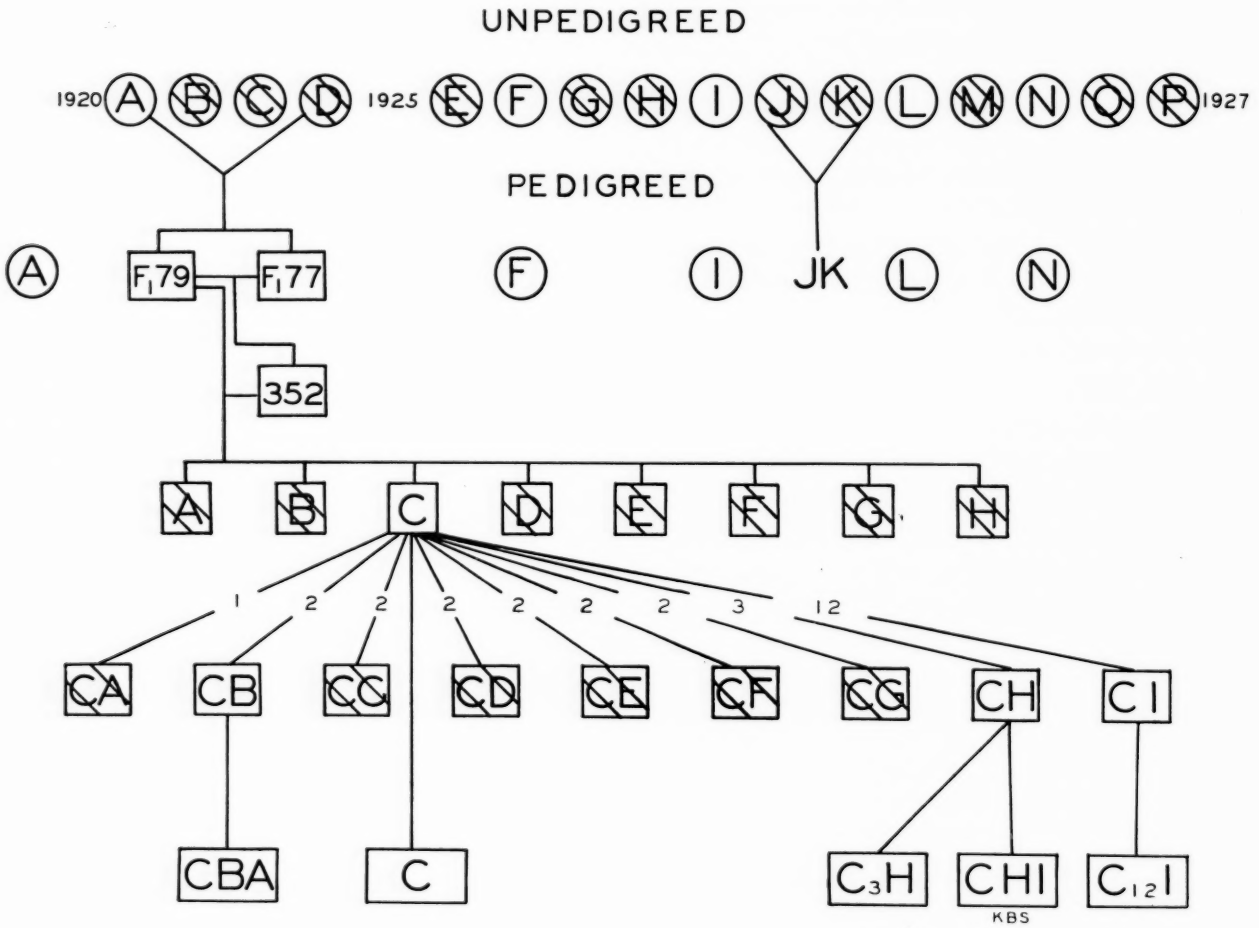

FIG. 1.—The origin of 11 inbred strains of mice and the genetic relationships existing between 6 of them. Those which had such genetic relationship are A, C, CBA, C3H, CHI, and C12I. Between 1920 and 1925, 4 groups of unpedigreed mice (A, B, C, D) were started on a brother-to-sister mating regime, thus becoming pedigreed; also between 1925 and 1927, 12 similar pairs of unpedigreed mice (E to P) were chosen; of these original 16 groups only 5 are still in existence. These are the A, F, I, L, and N. The JK strain was produced by a cross between mice of the original J and K strains. Following a cross between mice of the A and D strains, 5 selected strains were developed, the criterion of choice being the age distribution of spontaneous tumors of the mammary gland occurring in female mice. Crosshatching of a strain indicates that it is no longer in existence. The circles indicate stocks that are not related to each other. All strains in squares are to some extent genetically related to all other strains in squares, although many of them have been separated from each other for many years. The small numbers from 1 to 12 indicate the number of generations after the establishment of the C strain before the various sublines were established. For example the C12I strain was established from mice of the 12th generation. The first 5 strains were named because their coat colors were known by significant letters as follows: A, albino; B, black-eye white; C, cinnamon; D, dilute brown; and E, extreme dilution.

existence. The others (those crosshatched in the chart) have all died out from one cause or another: (a) sterility and other defects occurring in the early generations of inbreeding, (b) the hazards involved in moving mice from one laboratory to another, (c) intercurrent and unexpected diseases, primarily at those times when new mice were brought into the laboratory, and (d) expense. No inbred strain has ever been deliberately discontinued. The crosshatched D stock was developed from the trio of unpedigreed dilute brown mice referred to above. They were continued at St. Stephen's College for several years and a few descendants were given to Dr. C. C. Little at Cold Spring Harbor. All the dba or dbr strain are descended from this source. The original D strain established by the author in 1921 was continued at St. Stephen's College, Harvard University, University of Michigan, and Bar Harbor, Maine, but was lost shortly after the transfer of the mouse colony to New Haven. The unpedigreed A mice (one parent from Bagg albino, the other from an unpedigreed stock at the Carnegie Institute) were mated together and their descendants gave rise to the well-known A stock. As a distinguishing feature between mice of the Bagg albino and those of the A stock may be mentioned the fact that the incidence of spontaneous tumors of the mammary gland in the Bagg albino is extremely low, whereas in the A strain, which had been produced by selection toward a high incidence of spontaneous tumors of that gland, this particular neoplasm occurs frequently in breeder females. An individual of the A stock was crossed to a mouse of the D stock and produced mice Nos. 77 and 79. These 2 F_1's were mated *inter se* and produced male No. 352. Male No. 352 was then backcrossed to his mother, F_1 No. 79, and from this mating a large progeny was obtained, a few of which (1,668 female, 1,670 male, 1,672 male, 1,674 female, 1,507 female, and 1,510 female) are indicated in Fig. 2. This particular mouse (No. 79) has been described in more detail in another paper (28).

Originally the descendants of this mating were given the symbol HTF, signifying high tumor family, since numerous spontaneous tumors of mammary origin were found in them. It was soon apparent, however, that the biological variability indicated by analysis of the incidence of spontaneous tumors of the mammary gland (frequency or age distribution) warranted more than a single inbred line. Consequently sublines were established from time to time and continued by selection from that point onward. This was the origin of the C, C3H, CHI, CBA, and C12I inbred lines.

Thus was produced by genetic selection following hybridization a series of inbred sublines all related to each other to some extent at least, but differing among themselves in the incidence of spontaneous tumors of mammary origin. In sequence of cancer susceptibility these strains may be classified as follows: (a) C3H, (b) A, (c) C12I, (d) D, (e) C, (f) CHI, and (g) CBA. That is, selection following a cross between 2 original strains, A and D, with intermediate degrees of susceptibility to spontaneous cancer had given rise to divergent and extreme variants.

Fig. 2 presents the data on the serial number of the mice used in the production of these various sublines up to the point where the following strains were established: C, C3H, CHI, C12I, and CBA. Only those mice that were actually necessary for the production of the sublines are given. No mice in collateral lines are given in this chart.

Fig. 3 shows the pedigree of the first 53 generations of strain A; Fig. 4, similar data on the first generations of the C3H strain. The points at which the colony containing mice of these 2 strains was moved from one city to another are also given.

DISCUSSION

It is beyond the scope of the present paper to attempt a critical survey of the investigations that have been made possible in part by the use of these inbred strains of mice and their derived heterozygous individuals. Perhaps it would be of interest, however, to point out some of these contributions, especially in the field of cancer research. The original purposes for the establishment of these strains have been entirely fulfilled:

1. Both a statistical analysis of data obtained from the use of inbred mice and the practical attainment of reproducible results (*i.e.*, the supply of adequate controls) have shown on many occasions that in these mice biological variability has been reduced to a minimum or, at least, has been kept in an almost static condition. These data demonstrate conclusively, therefore, that the host factor in cancer and other biological research has been placed on quantitative grounds.

2. The supply of these mice has further emphasized the point that the application of genetic principles has had a tremendous influence on many phases of cancer research. These contributions may be briefly mentioned: (a) The use of these mice (A×D outcross shown in Fig. 1, together with derived F_2's, backcross and hybrid mice of the F_3 to F_7 generation obtained through selection) has demonstrated quite conclusively that the mechanism which determines susceptibility to the transplanted adenocarcinoma of the mammary gland is inherited according to mendelian principles. The final proof came with the establishment of mice whose response to the grafted tumor (susceptibility or resistance) was determined by one hereditary unit (gene) (29). (b) These

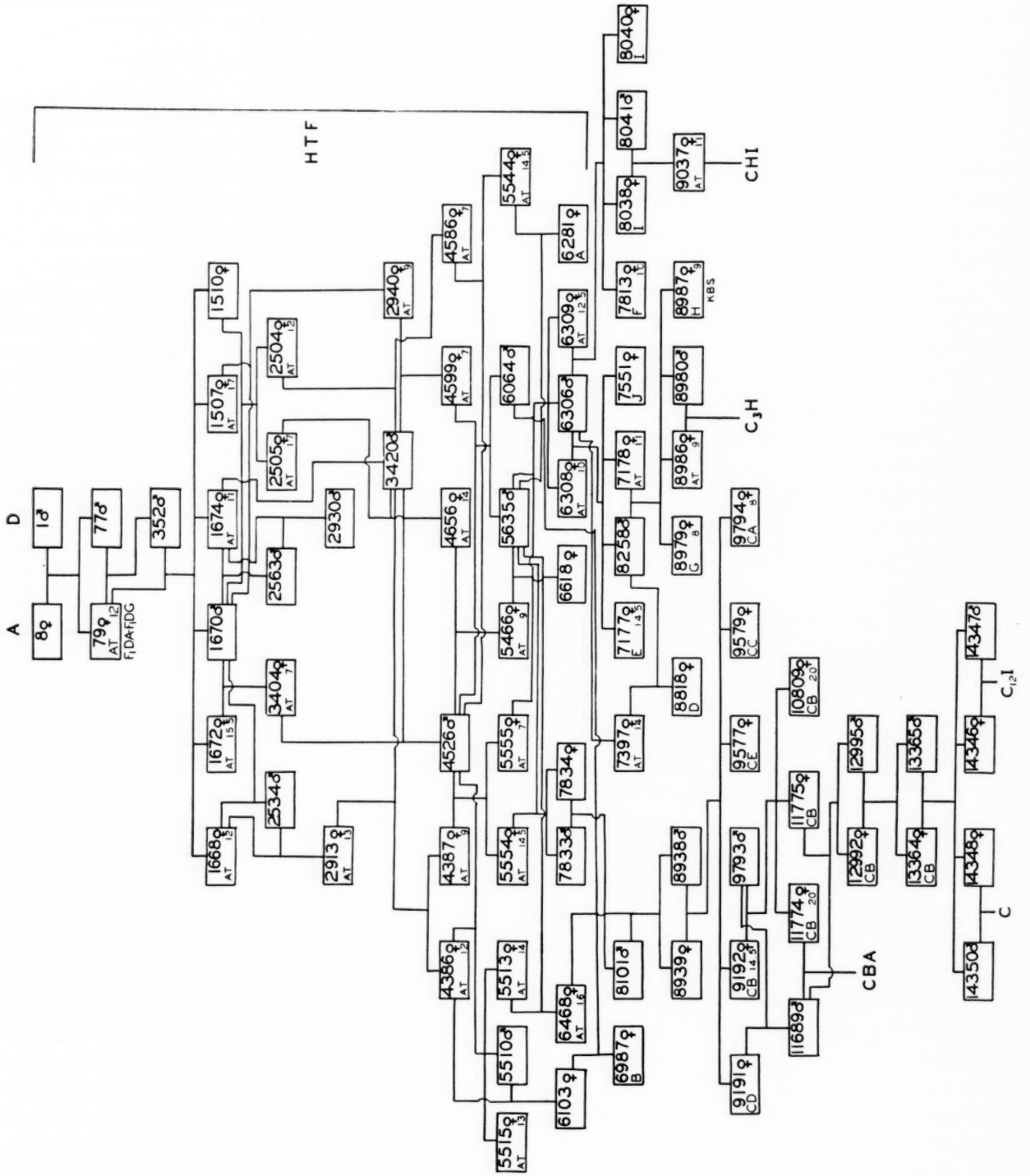

FIG. 2.—The actual interrelationships of individuals used in the development of the C, C3H, C12I, CHI, and CBA inbred strains of mice from a cross between mice of the A and D strains. Only those mice used in the production of these sublines are given. No collateral lines are represented. The symbol HTF at the right of the chart denotes the single high tumor family, referred to in the text, before the establishment of the 5 sublines C, C3H, C12I, CHI, and CBA. The small numerals in the lower right hand corners of the squares denote the age in months at which spontaneous tumors of mammary origin were obtained. When a significant symbol indicating the subline, such as A, B, C, etc., is not given in the lower left hand corners of the squares, the symbol AT is used. For example AT 14 means that a spontaneous tumor was found at 14 months of age. F1DA and F1DG are the symbols given to 2 transplantable adenocarcinomas of the mammary gland which had originated spontaneously in mouse F1 No. 79 at 12 months of age (28).

inbred mice supplied a wealth of spontaneous tumors of many kinds, and it was soon possible to determine that many of these types occurred not only frequently but also in a uniform or predictable fashion. That is, within a narrow margin the frequency distribution of spontaneous tumors did not fluctuate from time to time (30). This observation applied to adenocarcinoma of the mammary gland, adenocarcinoma of the lung (31), and to myelogenous and lymphatic leukemia (14). In addition to these commonest types

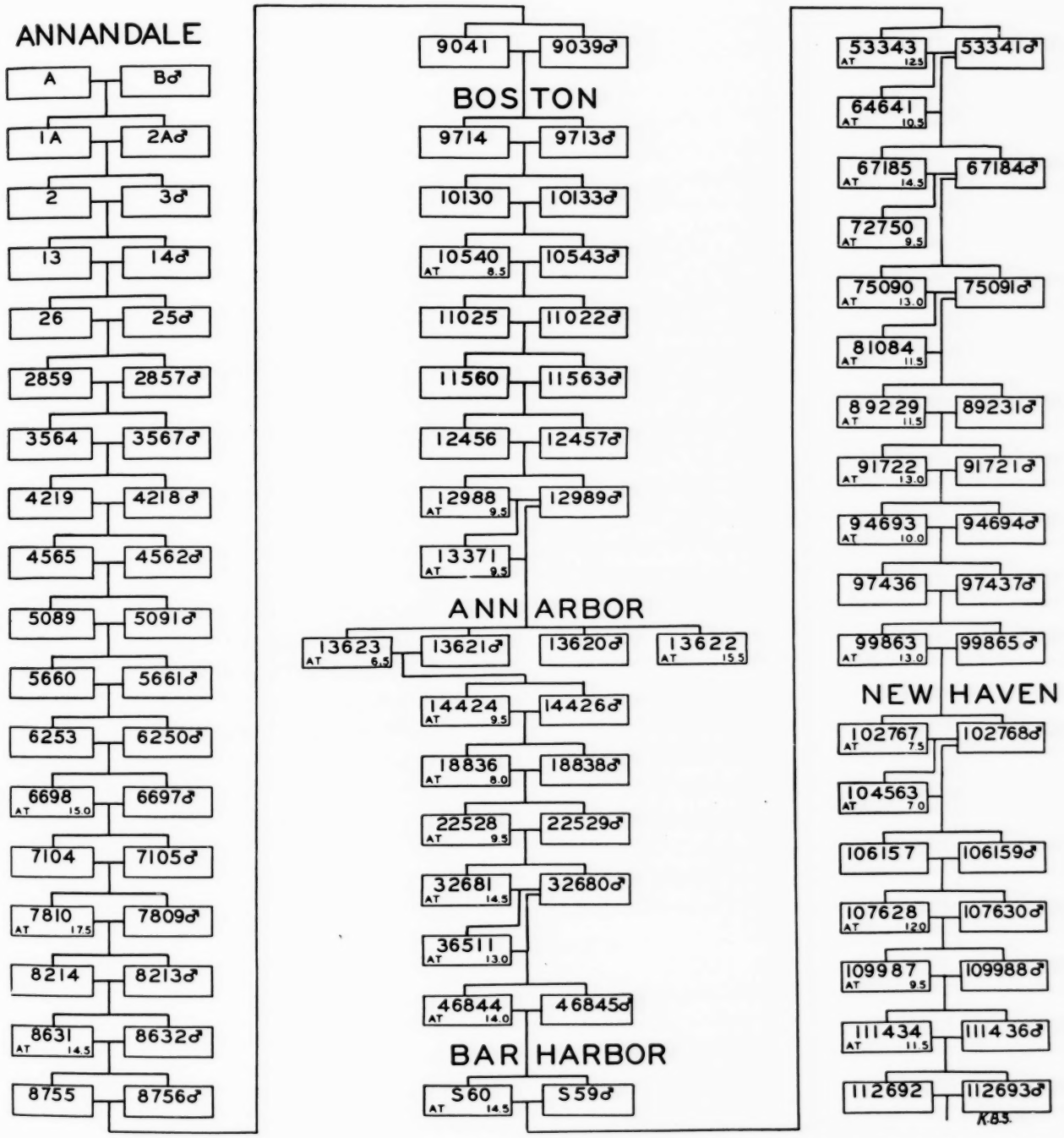

FIG. 3.—The pedigree of the first 53 generations of the A strain, showing the lineal descent of a single pair of mice obtained in 1921. In nearly every case the mating has been by a brother to his own sister. The direct female line is on the left, the male line on the right, except in case of mice Nos. 13623, 13621, 13620, and 13622, where the mice on the left and right are females and the 2 intermediately placed animals are males. The age at which spontaneous carcinoma of the mammary gland occurred is stated below the serial numbers of the females; for instance, AT 15.0 means that a spontaneous tumor appeared at 15 months of age. Where the space below the number of a mouse is blank, the mouse in question died of some other cause than cancer. The collateral lines containing thousands of mice together with several hundred instances of spontaneous cancer are not included in this chart. The times at which the A stock of mice was moved from one city to another are also given.

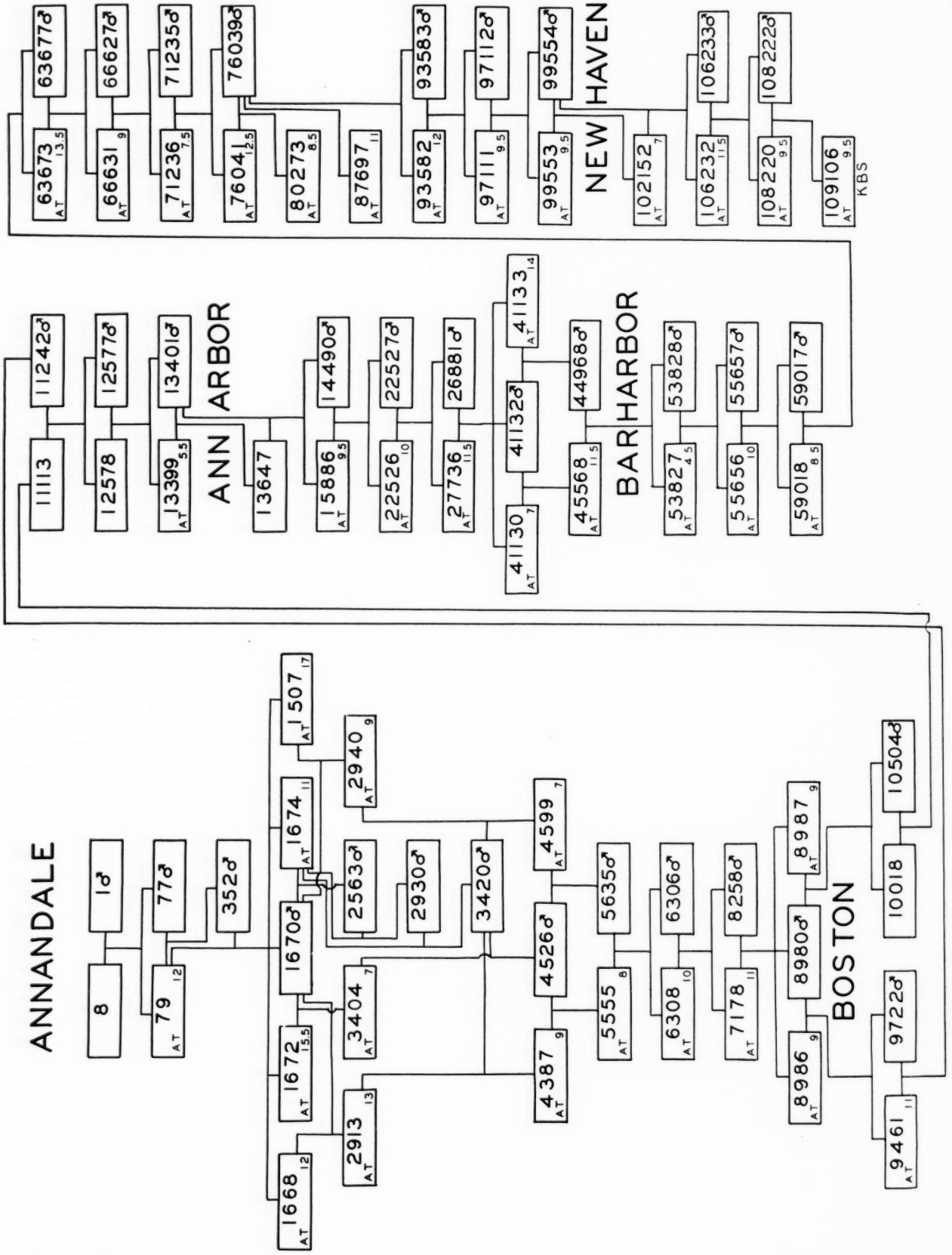

FIG. 4.—The origin of the C3H strain. The first 38 pedigreed generations are given. The males are indicated by the sign ♂. All other mice are females. The age in months at which spontaneous carcinoma of the mammary gland appeared is indicated below the serial numbers of the females. If the space is blank below the number of the female, the mouse died of some cause other than cancer. The times at which the C3H strain of mice was moved from one city to another are also given.

of tumors, however, others such as hepatoma (32–34), carcinoma of the ovary (35), and local sarcoma of the uterus (36) have been of rather frequent occurrence (although at uncertain times or unpredictably) in individuals of the CBA, CBA, and CHI strains respectively. (c) Another group of tumors also has occurred in mice of these inbred strains. These are, melanoma, small round cell sarcoma, lymphoblastoma (37), squamous cell carcinoma of anus, certain sporadic or isolated cases of adenocarcinoma of the mammary gland, adenocarcinoma of the duodenum, of the preputial gland (38), and of the cecum, and finally mixed cell tumors of the salivary glands (39). This group of tumors is of particular genetic interest, since they apparently arise sporadically and the mice that gave rise to them have been incapable of transmitting the tumor or susceptibility to these specific tumors to their descendants in direct line. They therefore have a nongenetic origin. (d) These mice have also provided control material for the analysis of that physiological state which is characterized by susceptibility or resistance to spontaneous tumors. These data have been adequately discussed at other times (10).

3. One other contribution of a genetic nature may be mentioned. The age distribution of spontaneous tumors of mammary origin in female mice of the various sublines mentioned in Fig. 1 and produced by selection has indicated that the difference between cancer and no cancer is probably only a quantitative (not a qualitative) difference (24). Since these mice were produced by the genetic principle of selection following a hybridization experiment and since, in this case, selection was effective in the establishment of statistically different sublines as far as the age distribution of spontaneous mammary cancer is concerned, the evidence is strong that genetic factors are involved in the origin of cancer of this gland.

The evaluation of genetic observations by other geneticists is complicated by the fact that mice from several sources have been employed. Of the two outstanding contributions to the genetics of cancer of the mammary gland in mice, one, that of Little with his collaborators at the Jackson Laboratory on extrachromosomal inheritance (20), was made possible by observations on the dba (a derivative of the D stock referred to in Fig. 1) and the C57 (a valuable inbred stock developed by Little working in collaboration with H. J. Bagg at Cold Spring Harbor). The source of the other strains reported (20) is not clearly indicated. According to Bittner (3), however, the Z stock is the C3H; the X the CBA,—direct descendants of mice of the C3H and CBA strains given to Bittner by the author in 1927 and 1928. The origin of the I strain is given in this paper. Thus of the 7 strains of mice used in the research on extrachromosomal inheritance, 5 were from the inbred strains established by Strong. The other contribution, that of Bittner on the "milk" influence (4–7) has been developed in a somewhat similar manner. In the early research on the "milk" influence, the C3H and A stocks (cancer-susceptible) were from the present author's source and the C57 (cancer-resistant) from the stock continued by Little.

The most fruitful field for the use of these inbred and their derived hybrid mice, aside from the genetic work outlined above, is perhaps in endocrinology. An analysis of these contributions has been made by Gardner (11). It is well, perhaps, to emphasize a few of those that have a particular genetic interest: (a) The incidence of carcinoma of mammary origin in male mice receiving estrogen parallels genetic susceptibility. This observation, originally made by Lacassagne (16) on inbred mice developed by Dobrovolskaia-Zavadskaia, has been verified by many investigators and particularly by Gardner and others (11) on mice of the various strains referred to in Fig. 1. (b) Gardner has also shown that presumably the same hormone treatment in mice of various inbred strains is followed by varying responses of several tissues or organs. These responses are moderately uniform within mice of a given strain and suggest fairly well that the fixation of different genetic constitutional mice through hybridization and selection may be an important factor in the result obtained by the injection of a specific hormone. Gardner has also found specific types of tumors, other than those of mammary origin, occurring in mice of various strains treated with various hormones. Most of these growths occur also in the F_1 as well as in the original strain, and may have a genetic basis. In the case of the adenoma of the pituitary gland the inheritance, although showing some dominance, may be of a complex nature. According to Gardner and Allen (12) carcinoma of the cervix may be obtained in female mice irrespective of genetic origin. Additional evidence, however, may indicate some genetic influence in this type of neoplasm also.

The field of the induction of tumors by carcinogens is beginning to show evidence that genetic factors influence the results obtained here also. Andervont (1) has demonstrated that the induction of carcinoma of the lung by a carcinogen parallels genetic susceptibility, thus verifying the conclusion of Lynch (17, 18) derived from the use of mice developed primarily by her. In fact, Andervont (2) maintains that there is a close parallelism between susceptibility to spontaneous lung tumors developed by genetic selection and susceptibility to the induction of tumors by the carcinogens. He further states that mice of the C3H strain (highly susceptible to spontaneous breast tumors) are more susceptible to the local subcutaneous induction of tumors with both 1,2,5,6-dibenzanthracene and methylcholanthrene than are mice of the Y strain (low susceptibility to the incidence of spontaneous tumors). Burdette and Strong (8) have obtained similar data using the C3H, CBA, CHI, NH, and JK strains.

Kirschbaum and his coauthors (15) have reported a similar situation in the induction of myelogenous and lymphatic leukemia in mice of the F strain (Fig. 1). These mice show a high spontaneous incidence of both types of leukemia as well as high susceptibility to the induction of both types of leukemia by a carcinogen. Strong (27) has recently reported that specific sublines of mice may be established following a hybridization and selection regime which gives rise to specific types of tumors induced by the same carcinogen (spindle cell sarcoma, squamous cell carcinoma of skin, rhabdomyosarcoma) or to no tumor whatever. Strong and Williams (40) have also shown that carcinoma of the mammary gland may be induced by methylcholanthrene in a mouse belonging to a strain made negative to spontaneous tumors of that tissue by selective genetic principles. That is, the carcinogen may replace genetic suscepibility.

Genetic strains have been employed by several investigators for the induction of tumors by the carcinogens. Branch (7a) reported that he was able to get almost twice as many tumors in C57 as in strain A by the injection of dibenzanthracene, thus indicating that there is no correlation between susceptibility to spontaneous tumors of mammary origin and susceptibility to skin tumors. Kreyberg (15a), working on selected lines de-

veloped by him, arrived at a general conclusion that spontaneous tumors of the breast and tar-induced tumors showed a definite segregation in different family lines—but an opposite distribution. Andervont, since 1934, has published extensively along similar lines. In one communication (2a) he reports observations on 8 established inbred strains as follows: C3H, C, C57, M, A, D, I, and Y. He concludes that "there are wide variations in susceptibility to both types of induced tumors. There is no correlation between the susceptibility to spontaneous mammary tumors and susceptibility to the induction of subcutaneous growths." Hence, "the conclusion may be drawn from the investigations that, up to the present time, a strain of mice has not been found which is resistant or susceptible to all types of tumor growth."

This challenge to genetics, expressed by Andervont, may be partly met after more of the inbred strains, many of which are mentioned in this paper, have been more fully investigated. If not, then the challenge should spur geneticists on, perhaps by a more critical application of the genetic principles of hybridization and selection, to the establishment of the ideal mouse, susceptible or resistant to all types of neoplasia.

SUMMARY

Data are presented showing the origin of 11 inbred strains of mice which are now being used in cancer and other biological research. Among these strains the A, C3H, CBA, and I are well known as indicated, partially, by the analysis of the use of mice in Hartwell's monograph on carcinogenesis (13). The others, the CHI, C12I, C, JK, F, L, and N have also been inbred for at least 17 years. It is shown that the A, C3H, CBA, CHI, C12I, and C are somewhat genetically related although they have been separated from each other by 20 years of inbreeding. The other strains are not related to any of these or to each other. A representative survey of the contributions that have been made possible, in part, by the use of these strains is also included.

REFERENCES

1. Andervont, H. B. Pulmonary Tumors in Mice. I. The Susceptibility of the Lungs of Albino Mice to the Carcinogenic Action of 1,2,5,6-Dibenzanthracene. Pub. Health Rep., **52**:212-221. 1937.
2. Andervont, H. B. The Production of Tumors in Mice of Strains C3H and Y by Dibenzanthracene and Methylcholanthrene. Pub. Health Rep., **53**:229-232. 1938.
2a. Andervont, H. B. Susceptibility of Mice to Spontaneous, Induced and Transplantable Tumors. Pub. Health Rep., **53**:1647-1665. 1938.
2b. Bagg, H. J. Individual Differences and Family Resemblances in Animal Behavior. A Study of Habit Formation in Various Strains of Mice. Arch. Psychol., **6**:1-58. 1920.
3. Bittner, J. J. The Genetics of Cancer in Mice. Quart. Rev. Biol., **13**:51-64, 1938.
4. Bittner, J. J. Further Studies on Active Milk Influence in Breast Cancer Production in Mice. Proc. Soc. Exper. Biol. & Med., **45**:805-810. 1940.
5. Bittner, J. J. Breast Cancer in Mice as Influenced by Nursing. J. Nat. Cancer Inst., **1**:155-168. 1940.
6. Bittner, J. J. The Influence of Foster Nursing on Experimental Breast Cancer. Tr. & Stud., Coll. Physicians, Philadelphia, 4 ser., **9**:129-143. 1941.
7. Bittner, J. J. Foster Nursing and Genetic Susceptibility for Tumors of the Breast in Mice. Cancer Research, **1**: 793-794. 1941.
7a. Branch, C. F. Dibenzanthracene Tumors in Controlled Strains of Mice. Am. J. Cancer, **26**:110-114. 1936.
8. Burdette, W. J., and Strong, L. C. The Incidence of Methylcholanthrene Induced Tumors in Inbred Strains of Mice. Genetics, **26**:143. 1941.
9. Cold Spring Harbor Symposia on Quantitative Biology. Vols. I-IX. Cold Spring Harbor, Long Island: The Biological Laboratory. 1933-1941.
10. Figge, F. H. J., Strong, L. C., Strong, L. C., Jr., and Shanbrom, A. Fluorescent Porphyrins in Harderian Glands and Susceptibility to Spontaneous Mammary Carcinoma in Mice. Cancer Research, **2**:335-342. 1942.
11. Gardner, W. U. Estrogens in Carcinogenesis. Arch. Path., **27**:138-170. 1939.
12. Gardner, W. U., and Allen, E. Cancer of the Cervix of the Uterus in Hybrid Mice Following Long-Continued Administration of Estrogens. Cancer Research, **1**:359-366. 1941.
13. Hartwell, J. L. Survey of Compounds Which Have Been Tested for Carcinogenic Activity. Federal Security Agency, U. S. Public Health Service. 1941.
14. Kirschbaum, A., and Strong, L. C. Leukemia in the F Strain of Mice: Observations on Cytology, General Morphology, and Transmission. Am. J. Cancer, **37**: 400-413. 1939.
15. Kirschbaum, A., Strong, L. C., and Gardner, W. U. Influence of Methylcholanthrene on Age Incidence of Leukemia in Several Strains of Mice. Proc. Soc. Exper. Biol. & Med., **45**:287-289. 1940.
15a. Kreyberg, L. The Genetic and Constitutional Aspects of Spontaneous and Induced Tumors. Univ. Wisconsin, Symposium on Cancer, pp. 3-19. 1938.
16. Lacassagne, A. Apparition de cancers de la mamelle chez la souris mâle, soumise à des injections de folliculine. Comp. rend. Acad. sc., **195**:630-632. 1932.
17. Lynch, C. J. Studies on the Relation between Tumor Susceptibility and Heredity. IV. The Inheritance of Susceptibility to Tar-Induced Tumors in the Lungs of Mice. J. Exper. Med., **46**:917-933. 1927.
18. Lynch, C. J. The Somatic Variability of Spontaneous Tumors. J. Cancer Research, **12**:318-325. 1928.
19. Russell, W. L. Inbred and Hybrid Animals and Their Value in Research. Chapter 10. In Snell, G. D. Biology of the Laboratory Mouse, Jackson Laboratory. Philadelphia: The Blakiston Company. 1941.
20. Staff of Roscoe B. Jackson Memorial Laboratory. The Existence of Non-Chromosomal Influence in the Incidence of Mammary Tumors in Mice. Science, **78**:465-466. 1933.
21. Strong, L. C. The Establishment of the "A" Strain of Inbred Mice. J. Hered., **27**:21-24. 1936.
22. Strong, L. C., and Hooker, C. W. Duplication of the Seminal Vesicles in Mice of the C Strain. Anat. Rec., **81**:333-349. 1941.
23. Strong, L. C. The Establishment of the C3H Strain of Mice for the Study of Spontaneous Carcinoma of the Mammary Gland. Genetics, **20**:586-591. 1935.

24. Strong, L. C. Production of the CBA Strain of Inbred Mice: Long Life Associated with Low Tumor Incidence. Brit. J. Exper. Path., **17**:60-63. 1936.
25. Strong, L. C. The Origin of the JK Strain of Inbred Mice. J. Hered., **28**:40-42. 1937.
26. Strong, L. C., Smith, G. M., and Gardner, W. U. Apparition anormale d'un épithélioma de l'anus chez une souris de race pure (souche F). Bull. Assoc. franç. p. l'étude du cancer, **25**:111-119. 1936.
27. Strong, L. C. A Genetic Analysis of the Induction of Tumors by Methylcholanthrene. With a Note of the Origin of the NH Strain of Mice. Am. J. Cancer, **39**: 347-349. 1940.
28. Strong, L. C. Transplantation Studies on Tumors Arising Spontaneously in Heterozygous Individuals. I. Experimental Evidence for the Theory That the Tumor Cell Has Deviated from a Definitive Somatic Cell by a Process Analogous to Genetic Mutation. J. Cancer Research, **13**:103-115. 1929.
29. Strong, L. C. The Genetic Basis of Susceptibility to Tissue Transplants. Proc. Nat. Acad. Sc., **12**:181-186. 1926.
30. Strong, L. C. Das Entstehungsalter von spontanen Brustdrüsenkrebsen bei weiblichen Mäusen (Muttertieren) des A-Stammes. Ztschr. f. Krebsforsch., **46**:272-284. 1937.
31. Strong, L. C. Unpublished data.
32. Strong, L. C., and Smith, G. M. Benign Hepatomas in Mice of the CBA Strain. Am. J. Cancer, **27**:279-284. 1936.
33. Strong, L. C., and Smith, G. M. Successful Transplantation of a Hepatoma in Mice. Am. J. Cancer, **28**:112-114. 1936.
34. Strong, L. C., and Smith, G. M. Preuve histologique de la présence de glycogène dans un hépatome malin transplantable. Bull. Assoc. franç. p. l'étude du cancer, **26**:694-698. 1937.
35. Strong, L. C., Gardner, W. U., and Hill, R. T. Production of Estrogenic Hormone by a Transplantable Ovarian Carcinoma. Endocrinology, **21**:268-272. 1937.
36. Strong, L. C. Unpublished data.
37. Strong, L. C. The Non-Genetic Appearance of Various Types of Neoplasia in Experimental Animals? J. Cancer Research, **12**:208-221. 1928.
38. Strong, L. C. The Transplantation of an Adenocarcinoma of the Preputial Gland in Mice of the A Strain. Cancer Research, **2**:332-334. 1942.
39. Strong, L. C. Four Rare Types of Spontaneous Tumors in Mice (Demonstration). Anat. Rec., **82**:99. 1942.
40. Strong, L. C., and Williams, W. L. A Genetic Analysis of the Induction of Tumors by Methylcholanthrene. III. Local and Remote Induction of Carcinoma of the Mammary Gland. Cancer Research, **1**:886-890. 1941.

Observations on the Genetics of Susceptibility for the Development of Mammary Cancer in Mice*†

J. J. Bittner, Ph.D.

(From the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine)

(Received for publication March 30, 1942)

As inbred strains of mice have been under observation for more than 50 generations of brother-to-sister matings, they should be satisfactory for genetic studies on the inherited susceptibility to cancer of the breast.

In an effort to determine how this susceptibility might be transmitted, reciprocal matings were made between mice of the high mammary tumor A and the low mammary tumor B (C57 black) strains. Breeding females of the B stock have an incidence of mammary tumors of 0.5 per cent as reported by Little, Murray and Cloudman (9); in the author's line no mammary tumors have been observed in several hundred mice. Only mice of one subline of the A stock, selected for a high incidence of mammary tumors, were used and they had an incidence of 96.8 per cent in a group of 527 mice. They represented mice of the 41 to 61 inbred generations. Preliminary reports have been published (4, 7).

All the hybrids considered in Table I nursed their mothers and were used as breeders. Those produced by mating females of the A strain to males of the B stock were called ABF_1 hybrids; hybrids resulting from the reciprocal cross were termed BAF_1. The mice of the 1st and 2nd generations were mated *inter se* to produce the animals of the 2nd and 3rd generations respectively.

The total number of hybrids descended from mothers of the low cancerous strain was 734 and in no generation did the incidence of mammary cancer exceed 2 per cent.

First generation hybrids with mothers from the cancer-susceptible strain and fathers from the low tumor strain had a high incidence of mammary tumors (ABF_1, 95 per cent). In mice of the ABF_2 generation the incidence was 76.3 per cent (300 mice).

TABLE I: INCIDENCE OF SPONTANEOUS CANCER OF THE MAMMARY GLAND IN BREEDING FEMALES OF THE A (HIGH CANCER) AND B (LOW CANCER) STRAINS AND THEIR F_1 TO F_3 HYBRIDS. ALL HYBRIDS NURSED THEIR MOTHERS

Strain of hybrid	Nursed by	Number	Cancer incidence, per cent	Average age: Cancer, months	Average age: Noncancer, months
A (F41–F61)	High cancer ♀	527	96.8	9.6	10.9
C57 black (B)	Low cancer ♀	568	0.5	21.4	20.8
ABF_1 (A♀ × B♂)	High cancer ♀	141	95.0	11.2	12.4
ABF_2	High cancer ♀	300	76.3	13.2	18.0
ABF_3	Cancerous F_2 mothers	285	68.8	12.1	18.5
	Noncancerous F_2 mothers	57	57.9	12.9	17.3
	Total	342	67.0		
BAF_1 (B♀ × A♂)	Low cancer ♀	148	1.4	12.5	21.7
BAF_2	Low cancer ♀	358	0.8	19.8	21.4
BAF_3	Low cancer ♀	228	0.9	19.0	21.1

The mice of the ABF_3 generation have been divided according to cancerous and noncancerous mothers of the F_2 generation. Those having cancerous mothers had an incidence of 68.8 per cent and the progeny of noncancerous mothers gave an incidence of 57.9 per cent. This difference was not statistically significant (1.6 × S. E.) as there were only 57 animals in the latter group. The incidence for the entire group of 342 ABF_3 mice was 67.0 per cent.

Preliminary data obtained by mating F_1 and F_2 females to males of the B strain are available. These mice are called backcross animals (BC) and since the cross was made to the B stock, the generations are termed the BBC generations. The prefix ABF_1, BAF_1, etc., designates the type of females used to make the cross.

* This investigation was aided by grants from the National Cancer Institute and The Jane Coffin Childs Memorial Fund for Medical Research.

† Read at the meeting of the American Association for Cancer Research, Boston, Massachusetts, March 31, 1942.

Many of the mice are still living but most of them have passed the cancer age and it is probable that only a few additional tumors will be observed (Table II). Those which had ABF_1 mothers have given an incidence of 53.3 per cent. The progeny of noncancerous ABF_2 mothers have had an incidence of 5 per cent; those with cancerous mothers an incidence of 47.4 per cent. The incidence of mammary tumors recorded in the BAF_1-BBC and BAF_2-BBC generations was 0.5 and 1.5 per cent respectively for a total of 496 mice. The living mice are from 17 to 28 months of age.

To obtain mice of the hybrid generations it is possible either to use a large number of mothers and to mate a small number of progeny from each female or to use a small number of mothers and secure as many young as possible from each animal. The latter method was used in this work as it gives a greater opportunity to study the makeup of the individual parents. From 15 to 20 mothers were used in each group and the average number of female progeny observed from each was 18.

The incidence of tumors in the hybrids was greater for mice born in the 3rd and succeeding litters than for members of the 1st and 2nd litters. The data are represented graphically in Fig. 1 for the ABF_1, ABF_2, and ABF_3 generations. Only mice having 8 or more progeny are included in the tabulation by litters and the number of mice in some groups may differ from the figures given in Table I.

To determine further if the age at which the mothers developed tumors might have any effect on the incidence of mammary tumors in their progeny, the F_2 and F_3 data were arranged as presented in Table III and Fig. 2. The 962 mice include hybrids of the ABF_2, ABF_3, BAF_2, and BAF_3 generations nursed by females of the A stock. The BA groups are not given in Table I as they were not used to make BBC hybrids, but they have been reported elsewhere (7).

The mice whose mothers developed tumors when they were from 200 to 300 days of age had a higher incidence of mammary tumors than did the young of mothers which developed tumors at later ages. These differences were not significant. In every group, however, the incidence was higher in the mice born in the 3rd and following litters than for those of previous litters, and in every group the difference was significant (Table III).

To ascertain if fathers of the C57 black strain might exert some influence, the mice of the fostered BAF_1 generation were considered. They had mothers of the B stock, fathers from the A stock, and were nursed by females of the A strain. They are tabulated accord-

TABLE II: INCIDENCE OF MAMMARY CANCER IN ANIMALS OF THE BACKCROSS GENERATIONS MADE BY MATING HYBRID FEMALES TO MALES OF THE B (C57 BLACK) STOCK

Mating	Nursed by	Number	Incidence, per cent	Living, per cent	Age of living animals, months
ABF_1–BBC ..... ABF_1 ♀ × B♂	High cancer ♀	330	53.3	27.6	12–24
ABF_2–BBC ..... ABF_2 ♀ × B♂	Cancerous F_2 mothers	234	47.4	38.9	
	Noncancerous F_2 mothers	60	5.0	78.3	
	Total	294	38.8	46.9	12–23
BAF_1–BBC ..... BAF_1 ♀ × B♂	Low cancer ♀	221	0.5	38.0	18–28
BAF_2–BBC ..... BAF_2 ♀ × B♂	Low cancer ♀	275	1.5	38.9	17–27

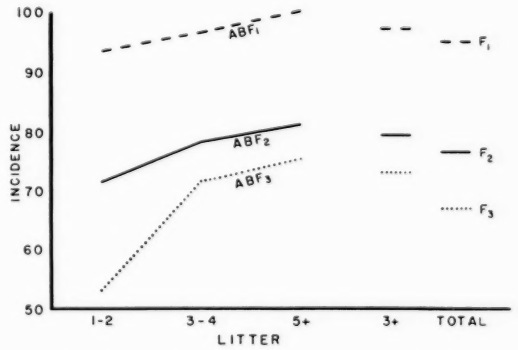

FIG. 1.—Incidence of mammary cancer in hybrid mice, tabulated by successive litters.

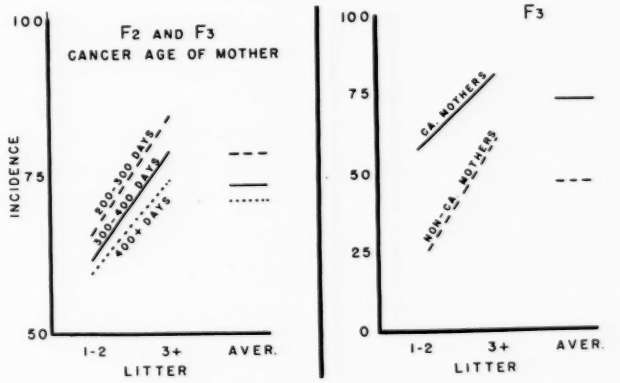

FIG. 2.—Incidence of mammary cancer in hybrids of the F_2 and F_3 generations born in successive litters, according to the age at which their mothers developed tumors, and in mice of the F_3 generations with cancerous or noncancerous F_2 mothers.

ing to the litters in which they were born to these B stock females and to the litters thrown by females of the A stock before they were used as foster mothers (Table IV).

The mice nursed by females of the A strain following their first litters gave an incidence of 78.6 per cent and those nursed by females following their 3rd litters had an incidence of 95.9 per cent. There was little variation in the mice born in successive litters to females of the B stock. The difference in incidences was 17.3 per cent and may possibly be statistically significant (2.0 × S. E.). The incidence for the entire group (89.7 per cent) was intermediate to those given above.

For further comparisons the data obtained from observations made on the mice of the ABF_3 by A female and BAF_3 by A female generations were tabulated (Table V and Fig. 3). The former group was descended from matings between A female and B male and the latter resulted from the cross between B female and A male. The mice of each group were descended from 1st generation animals which nursed females of the high mammary cancerous A stock. The number of mice was 322 and 272 and the incidence of mammary tumors 69.3 and 68.4 per cent respectively. Also there was no significant difference in the mice born in different litters.

The difference in incidence of tumors between the ABF_3 hybrids born in the 1–2 and 3+ litters was 22.8 per cent; for the BAF_3 mice the difference was 32.2 per cent. These differences are significant.

TABLE III: INCIDENCE OF MAMMARY CANCER IN ABF_2, BAF_2, BAF_3, AND ABF_3 HYBRIDS NURSED BY FEMALES OF THE A STRAIN, TABULATED BY LITTERS ACCORDING TO THE AGE AT WHICH THE MOTHERS DEVELOPED TUMORS, OR BY NONCANCEROUS F_2 MOTHERS

Cancer age of mothers, days	1–2 litters		3+ litters		Total		Difference in incidence between 1–2 and 3+ litters
	Number	Cancer, per cent	Number	Cancer, per cent	Number	Cancer, per cent	
200–300	74	66.2	139	84.9	213	78.4	18.7% or 3.2 × S. E.
300–400	115	64.3	199	78.9	314	73.6	14.6% or 2.8 × S. E.
400 +	92	59.8	236	76.3	328	71.6	16.5% or 3.0 × S. E.
Total	281	63.3	574	79.3	855	74.0	16.0% or 5.0 × S. E.
Cancerous F_2 mothers making F_3	154	57.8	333	81.1	487	73.7	23.3% or 5.4 × S. E.
Noncancerous F_2 mothers making F_3 hybrids	43	25.6	64	60.9	107	46.7	35.3% or 3.6 × S. E.
Total	324	58.3	638	77.4	962	71.0	19.1% or 6.2 × S. E.

TABLE IV: INCIDENCE OF MAMMARY CANCER IN BAF_1 MALE BY A FEMALE HYBRIDS, TABULATED ACCORDING TO LITTERS BORN TO THEIR MOTHERS (B STOCK) AND FOSTER MOTHERS (A STOCK)

BAF_1 by A ♀ — Nursed by females of the A stock following their

Born to B ♀ in litter	1st litter		2nd litter		3+ litter		Total	
	Number	Cancer, per cent	Number	Cancer, per cent	Number	Cancer, per cent	Number	Cancer, per cent
1	27	77.8	23	78.3	22	95.5	72	83.3
2	5	80.0	15	93.3	3	100.0	23	91.3
3+	10	80.0	20	100.0	24	95.8	54	94.4
Total	42	78.6	58	89.7	49	95.9	149	88.6

TABLE V: INCIDENCE OF MAMMARY CANCER BY LITTERS, IN MICE OF THE ABF_3 MALE BY A FEMALE AND BAF_3 MALE BY A FEMALE GENERATIONS. ONLY THE PROGENY OF MOTHERS HAVING 8 OR MORE YOUNG ARE TABULATED

		Cancer incidence by litters						
		1–2 litters		3+ litters		Total		Difference in incidence between 1–2 and 3+ litters
		Number	Cancer, per cent	Number	Cancer, per cent	Number	Cancer, per cent	
ABF_3 by A ♀	Cancerous and noncancerous	84	51.2	238	73.5	322	67.7	22.3% or 3.8 × S. E.
BAF_3 by A ♀	F_2 ♀♀	116	46.6	162	78.4	278	65.1	31.8% or 5.5 × S. E.
Total F_3	Cancerous F_2 ♀♀	157	54.8	336	78.3	493	70.8	23.5% or 6.4 × S. E.
Total F_3	Noncancerous F_2 ♀♀	43	25.6	64	60.9	107	46.7	35.3% or 3.6 × S. E.
Total F_3		200	48.5	400	75.5	600	66.5	27.0% or 6.6 × S. E.

DISCUSSION

As stated in previous publications there are intrinsic and extrinsic influences operating in the development of mammary cancer in mice (4). Those which have been recognized are: (a) genetic susceptibility, (b) hormonal stimulation, and (c) an active milk influence obtained while nursing from females of stocks having a high incidence of mammary tumors.

Susceptibility to the development of mammary cancer may be transmitted by males and females of the susceptible strain and is an intrinsic factor. Shimkin and Andervont (10) found that the amount of estrogenic hormones secreted by mice of various strains was not causally related to the formation of mammary cancer but was due to strain differences. This might

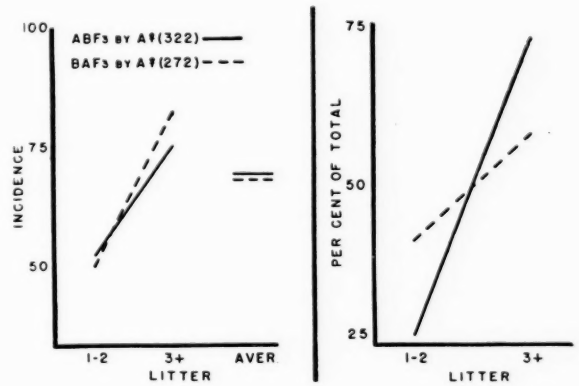

FIG. 3.—Incidence of mammary tumors by litters, in hybrids of the ABF_3 by A female and BAF_3 by A female generations and the percentage of the total of each group born in the respective litters.

be considered as resulting from intrinsic causes but others must be considered.

In a strain of mice with a high incidence of mammary tumors in breeding and virgin females (1) the amount of estrogenic hormones needed for the development of these tumors may result from intrinsic factors. In other strains, the A stock for example, the amount produced as the result of intrinsic factors is not sufficient to elicit tumors in more than 5 per cent of the virgin females. If hybrids are produced by mating females of the A stock to males of the C57 black stock, the hybrid females maintained as virgins have the same incidence of mammary tumors as the virgin females of the A stock (3). The increased amount associated with the production of young is necessary before a high incidence of tumors will be observed. In such a stock the extrinsic causes (breeding) of hormonal stimulation would have to supplement the amount produced by intrinsic factors before tumors would be expected. In other strains breeding plays a role in causing the tumors to appear at an earlier age (1).

The influence normally obtained in the milk of females of mammary cancer strains is an extrinsic cause in the production of mammary tumors, as it may be eliminated entirely by foster nursing (5). An active influence may also be supplied by feeding or injecting extracts containing this influence, and the amount thus obtained may determine the incidence of tumors and the age at which they appear.

Few spontaneous mammary tumors occurred in hybrids with mothers from a strain of mice with a low incidence. Mice of 3 hybrid and 2 backcross generations were observed (1,230 mice). These mice would not obtain the active milk influence.

In the reciprocal cross (mothers from a cancerous and fathers from a low cancerous stock) the incidence of tumors recorded in hybrids of the 1st and 2nd generations is in accord with the genetic theory that susceptibility to mammary cancer is inherited as a single dominant factor. Preliminary data secured in the backcross generations to males of the low cancer stock also support this theory.

After mating females of the 2nd hybrid generation to produce mice of the 3rd hybrid generation it was noted that the progeny of *cancerous* females had a higher incidence than did the progeny of *noncancerous* mothers. *Noncancerous* F_2 females mated to males of the resistant strain had progeny with a very low incidence of tumors; the incidence of cancer among the progeny of *cancerous* F_2 females was higher, as would be expected according to the theory.

To determine the number of factors involved for susceptibility to mammary cancer, the incidences observed should be compared with theoretical incidences which would be expected for different numbers of factors. The application of statistical methods should then enable one to determine the genetic constitution of the character being studied.

In the present investigation it is impossible to make these comparisons with any degree of accuracy because, in the hybrids, different incidences of tumors were observed in the mice of successive litters. In every group the incidence in the 1st and 2nd litters was less than that for mice born in the following litters. The incidence for the total number in each generation represents a figure intermediate between the incidence as tabulated by litters and may or may not be a true incidence for the entire group unless the mice are equally distributed by litters.

From a genetic standpoint there is no reason to expect that the mice born in successive litters would differ in their susceptibility to the development of carcinoma of the mamma. Also there was no evidence in our hybridization cross that the father exerted any influence, as indicated by Andervont (2).

There is some evidence, however, that the age at

which a female develops a tumor may have some bearing on the incidence of tumors to be expected in her progeny. The younger the mother at the time she develops the tumor, the higher will be the incidence in her daughters. The average age at which the young developed tumors has not been tabulated.

The only explanation we can advance for these findings is that they may be due to different concentrations of the active milk influence in mice of various ages. If we assume that the active influence present in the milk of females of cancerous stocks may be, as has been suggested (6, 11), a virus, this influence would have the ability to multiply with the increasing age of the mice. Thus we might expect that mice born in the first litters would obtain a smaller amount than those born in the following litters. The incidence of mammary tumors would be associated with the amount of the influence received.

While the data observed in reciprocal hybrids indicate that susceptibility to mammary tumors may be a single factor, different incidences observed in successive litters of mice and/or the age at which the mothers develop tumors make it impossible to state the exact nature of the susceptibility to spontaneous mammary cancer. That there is an inherited susceptibility has been demonstrated by various workers and has been substantiated in these studies. The observation of different incidences of mammary tumors among the progeny of *noncancerous* females of the 2nd hybrid generation when mated to males of the same generation or males of the resistant strain is of importance in demonstrating this susceptibility.

Until a few years ago mammary tumors were believed to result from a combination of estrogenic stimulation and genetic susceptibility. After it was demonstrated that an extrachromosomal (milk) influence was involved, the role of inherited susceptibility was said to be of minor consequence. With the use of larger numbers, and as the result of observations on hybrid mice, the significance of the genetic constitution of the host was again recognized. In the meantime, however, a few workers have neglected to mention the function of estrogenic hormones in preparing mammary tissue so the cancerous change may take place.

Most workers are of the opinion that inherited susceptibility for mammary tumors is dominant, but the use of different inbred strains of mice has not resulted in a uniform theory regarding the exact constitution of this susceptibility. This is what we might expect, as it has been noticed many times that the same strain of mice may not show the same incidence of tumors in different laboratories and that the same strain may even show different incidences in the same laboratory at various times. No genetic comparison has been made with the same strains of mice in hybrid crosses.

Using females from a single strain of high mammary cancer mice and males from different strains with a low incidence, Andervont (2) concluded that the fathers exerted some influence on the susceptibility to these tumors and that genetic factors exerted their influence by controlling the degree of susceptibility. The data secured from crosses between the C_3H females and males of the I and C57 black stocks would support the theory for a single factor; those obtained with males of the Y (yellow) strain would not. It is of interest that Little (8) observed in a cross between dilute brown and yellow strains that the yellow mice had a significantly lower incidence of mammary tumors than did the nonyellow mice. This may account for the variations observed by Andervont.

Since the mothers in Andervont's experiments were members of the same inbred strain, their contribution to the inherited susceptibility for mammary tumors should have been the same regardless of the fathers. It is possible, however, that the male parents contributed factors which modified susceptibility transmitted by the females of the cancer-susceptible strain.

Another possibility, suggested by the work of Shimkin and Andervont (10), is that intrinsic factors transmitted by the fathers may have produced different levels of estrogenic secretion in their hybrid female offspring. The hybrids secreting the smallest amount of hormones would be expected to develop tumors later and to have the lowest incidence of mammary tumors. Andervont observed that hybrids with the lower incidences developed tumors at a later age than those with higher incidences.

In general the results reported by Andervont and the data given above agree except for minor details, as might be expected with the use of different stocks in different laboratories.

CONCLUSIONS

Data obtained on the incidence of mammary tumors in hybrid mice show that inherited susceptibility is transmitted as a dominant.

The results are in accord with the genetic theory that it is inherited as a single factor.

Mice which develop tumors at an early age have progeny with a higher incidence of mammary tumors than mice born to mothers which develop their tumors at later ages.

The incidence of mammary cancer recorded among the progeny of *cancerous* females is higher than the incidence for the progeny of *noncancerous* females of the second hybrid generation.

That the milk influence may be more concentrated

in older mice is suggested by the incidence of tumors when the young are tabulated by litters.

REFERENCES

1. Andervont, H. B. Spontaneous Tumors in a Subline of Strain C3H Mice. J. Nat. Cancer Inst., **1**:737-744. 1941.
2. Andervont, H. B. The Influence of the Paternal Parent in Determining the Susceptibility of Mice to Spontaneous Mammary Tumors. J. Nat. Cancer Inst., **2**:7-11. 1941.
3. Bittner, J. J. Breast Cancer in Breeding and Virgin "A" and "B" Stock Female Mice and Their Hybrids. Pub. Health Rep., **54**:1113-1118. 1939.
4. Bittner, J. J. "Influences" of Breast-Cancer Development in Mice. Pub. Health Rep., **54**:1590-1597. 1939.
5. Bittner, J. J. Foster Nursing and Genetic Susceptibility for Tumors of the Breast in Mice. Cancer Research, **1**:793-794. 1941.
6. Bittner, J. J. The Preservation by Freezing and Drying in Vacuo of the Milk-Influence for the Development of Breast Cancer in Mice. Science, **93**:527-528. 1941.
7. Bittner, J. J. The Influence of Foster Nursing on Experimental Breast Cancer. Tr. & Stud., Coll. Physicians, Philadelphia, **9**:129-143. 1941.
8. Little, C. C. The Relation of Coat Color to the Spontaneous Incidence of Mammary Tumors in Mice. J. Exper. Med., **59**:229-250. 1934.
9. Little, C. C., Murray, W. S., and Cloudman, A. M. The Genetics of Non-Epithelial Tumor Formation in Mice. Am. J. Cancer, **36**:431-450. 1939.
10. Shimkin, M. B., and Andervont, H. B. Effect of Foster Nursing on the Response of Mice to Estrogens. J. Nat. Cancer Inst., **1**:599-605. 1941.
11. Visscher, M. B., Green, R. G., and Bittner, J. J. Characterization of Milk Influence in Spontaneous Mammary Cancer. Proc. Soc. Exper. Biol. & Med., **49**:94-96. 1942.

The Irradiation of Transplanted Bagg-Jacksen and Yale Carcinomas in Mice, as Affected by Diet and Foster Nursing*

Jules A. Plaut, M.D.,** Robert Tennant, M.D., and Ashley W. Oughterson, M.D.

(From the Departments of Surgery and Pathology, Yale University School of Medicine, New Haven, Conn.)

(Received for publication March 30, 1942)

It is common clinical experience that tumors do not respond uniformly to x-ray. Some of the factors associated with this varied response are known (Stewart and others). It is well established that the cell type, size, and location of a tumor are important considerations in determining its radiosensitivity, although even these factors do not appear to be invariably reliable. While attention has been directed chiefly to the morphology, size, and location of the tumor, it has become apparent that there are other more important and still unknown factors which determine the radiosensitivity.

Previous experiments (6, 8) indicate that the host or the interaction of host and tumor play an important role in determining the response of the tumor to irradiation. It was found that when a malignant mammary adenocarcinoma was transplanted into the parent strain (A albino) and treated with a single dose of 2,500 r no cures were obtained. When the same tumor was transplanted into an F_2 hybrid (AC57) 80 per cent cures were obtained. In both instances the tumor took in 100 per cent of the animals. In the parent stock there were no regressions. The conclusion from these experiments was that x-ray augmented the natural resistance of the host. Whatever the host-tumor relation may have been, it appeared that x-ray brought to light these latent factors.

The present experiments were designed to extend these observations and determine whether other factors said to influence the development of tumors or their growth would also change the response of the tumor to x-ray. The following experiments were planned.

One of the objections to postulating increased radiosensitivity of the tumor in the F_2 generation was that some type of foreign protein reaction might be responsible. Thus the summation of x-ray damage plus such a foreign protein reaction might yield a higher cure rate. It is well known that tumors take and grow in 100 per cent of the animals when transplanted into the F_1 generation. Furthermore normal tissue transplants behave in the F_1 generation as in the parent stock. It appeared, therefore, as though a tumor transplanted into the F_1 hybrid should respond to x-ray as it does in the parent stock.

Bittner's (5) work with inbred strains of mice has shown that the incidence of spontaneous mammary tumors depends on the presence of 3 factors. These are: (a) a genetically inherited breast cancer susceptibility, (b) a breeding or hormonal factor, and (c) a breast cancer-producing influence transmitted in the milk of high cancer stock mothers. Previous experiments have demonstrated that the F_1 generation contains all the necessary genetic factors for transplantation of a tumor arising in either parent host. A tumor arising in either parent host will take in 100 per cent, does not regress, and grows in a manner comparable to that in the parent but at a somewhat increased rate. By using a transplanted tumor, rather than a spontaneous growth, one can disregard the hormonal factor. Hence by foster nursing the milk factor alone may be studied.

The experiments of Strong (9, 10), Bittner (2–4), and others have demonstrated that the behavior of inbred strains of mice differs depending on the diet. This effect is evidenced by alterations in the life span, size, and vigor of the animal, and the age at which spontaneous tumors appear. Diet affects the size of litters and even the sex ratio of those litters. There is thus abundant experimental evidence to suggest that diet may influence the behavior of tumors themselves. Moreover, since breast milk contains a factor indispensable for the development of a tumor, is it not reasonable to assume that a mother's diet may alter the constitution of her milk and thus perhaps affect the incidence and possibly the characteristics of tumors in her offspring?

These experiments were therefore designed to determine whether or not diet and the milk factor influ-

* This investigation was aided by grants from The Jane Coffin Childs Memorial Fund for Medical Research and The Anna Fuller Fund.

** National Cancer Council Fellow.

enced the growth characteristics or the x-ray response of a transplantable tumor in the F_1 generation.

METHODS AND MATERIALS

The animals used were the first generation hybrids obtained by crossing strain A albino and C57 black mice. At the time of mating some parents were put on one diet, and some on another. Both parents of any particular litter ate the same diet. When a litter was born it was allowed to suckle for 4 weeks, then weaned to the parents' diet, on which it was maintained until the end of the experiment.

Half the newborn were foster nursed and half allowed to suckle their own mothers. Thus half the F_1 hybrids obtained strain A milk, and half had C57 black milk. All the fostering was done within 24 hours after birth, and usually within 12 hours.

Thus, within a diet group, there were ABF_1-A, ABF_1-B, BAF_1-A, and BAF_1-B hybrids. In this designation, the initial letter denotes the mother's strain and the final letter indicates the source of milk.

The diets were commercial products currently used in many laboratories. They will be designated by the numbers 1 and 2, and analyzed as follows:

Diet 1 (Purina Fox Chow)

	Per cent
Crude protein not less than	20.0
Crude fat not less than	3.0
Crude fiber not more than	6.0
Nitrogen-free extract not more than	46.0

Ingredients: Flavin concentrate, carotene, wheat germ, dried skim milk, liver meal, brewer's dried yeast, barley malt, fish meal, dried meat, alfalfa meal, corn grits, soy bean oil meal, cereal feed (from corn and wheat), molasses, dried beet pulp, cod liver oil, 1 per cent steamed bone meal, and 1 per cent iodized salt.

Diet 2 (Nurishmix)

	Per cent
Crude protein not less than	20.0
Crude fat not less than	6.5
Crude fiber not more than	3.5
Carbohydrates	51.5
Nitrogen-free extract not less than	48.0

Ingredients: Dried buttermilk, beet scrap, wheat germ meal, rolled oats, molasses, 1 per cent iodized salt, U.S.P. cod liver oil.

The mice were given unlimited access to the food pellets, which were placed in a hopper at the end of each cage. Water was unrestricted. No other food was given. The mice were kept in galvanized tin cages, on wood shavings. From 2 to 4 weeks after weaning each mouse was inoculated with 2 separate tumors, with an interval of 24 to 48 hours between the 2 transplantations. The latter was done in the following manner:

A healthy looking Yale tumor, described by Gardner, Strong, and others (7), was ground in a Latapie apparatus and 0.03 cc. of a saline suspension of this was injected subcutaneously into the outer aspect of the left thigh. The next day, or the day after, 0.03 cc. of a saline suspension of the Bagg-Jacksen tumor (1) was inoculated into the lateral aspect of the right thigh. Sometimes the Bagg tumor was inoculated first, but more often it followed. The Yale tumor is a breast carcinoma originally induced by theelin in a strain A mouse. The Bagg-Jacksen tumor is a mammary adenocarcinoma produced in C57 blacks by forced breeding.

The tumors were irradiated when their diameters measured about 5 to 8 mm. (usually 7 to 10 days after inoculation). This was done under light seconal anesthesia. The x-ray dose was delivered in one exposure of 2,500 r. The radiation factors were 90 kv., 4 ma., 23.5 cm. distance, no filtration, size of port 2 cm. cone. The output was 149 r per minute. The tumors were measured in 2 diameters on the day of radiation and afterwards on the 4th, 7th, 10th, and 14th day and thereafter at weekly intervals until 16 weeks postirradiation. As each animal died it was autopsied and sections of each tumor were taken for histological study. Those few animals living 16 weeks after the second tumor was x-rayed were killed and autopsied.

Control tumors were not radiated.

The animals were divided for comparison into 8 categories depending on the diet, breast milk, and parentage. Each group contained about 50 experimental and 10 to 20 control mice.

Cured animals are those dead or killed at the end of 4 months with no microscopic evidence of tumor.

RESULTS

Effect of Diet on Cure Rates

There was no consistent significant difference in the behavior of the tumors which could be referred to the diets. When mice are raised on diet 1 they themselves grow to be larger and heavier, are more active, and have a shorter life span than those fed diet 2. But transplanted, radiated tumors in these animals follow no consistently divergent patterns. With the Bagg tumor we found that in 2 groups there were more cures with diet 1; in one group there were more cures with diet 2; and in the remaining group the cures were about the same. With the Yale tumor, diet 1 produced more cures in one group, while in the other 3 the cures were about alike. Thus there were no significant differences (Table III).

Behavior of the Bagg Tumor Compared to That of the Yale Tumor

The Bagg tumor is an adenocarcinoma composed mainly of small acini and to a lesser extent of sheets

TABLE I: INFLUENCE OF MILK FACTOR
CURES
2,500 r; Combined Diets

	Cured	Not cured	Total	Per cent
YALE TUMOR				
A mothers, B fathers, A milk	21	69	90	23.3
A mothers, B fathers, B milk	24	76	100	24.0
B mothers, A fathers, A milk	21	85	106	19.8
B mothers, A fathers, B milk	36	63	99	36.4
BAGG-JACKSEN TUMOR				
A mothers, B fathers, A milk	49	43	92	53.2
A mothers, B fathers, B milk	51	49	100	51.0
B mothers, A fathers, A milk	47	61	108	43.5
B mothers, A fathers, B milk	46	50	96	47.9
OVER-ALL AVERAGE CURE				
Yale tumor				25.9
Bagg tumor				48.9

TABLE II: INFLUENCE OF MILK FACTOR
CONTROL REGRESSIONS
No Radiation; Combined Diets

	Regressed	Dead with tumor	Total	Per cent
YALE TUMOR				
ABF_1-A	5	18	23	21.7
ABF_1-B	1	34	35	2.9
BAF_1-A	1	37	38	2.6
BAF_1-B	0	29	29	0.0
BAGG TUMOR				
ABF_1-A	6	19	25	24.0
ABF_1-B	5	30	35	14.3
BAF_1-A	1	35	36	2.5
BAF_1-B	5	27	32	15.6

TABLE III: EFFECT OF DIET
RADIATED TUMORS

		No. treated	* Not counted	Net total	Dead with tumor	Cured
DIET 1						
ABF_1-A:	Bagg	51	6	45	24	21(46.7%)
	Yale	48	6	42	32	10(23.8%)
ABF_1-B:	Bagg	47	0	47	20	27(57.4%)
	Yale	46	0	46	34	12(26.1%)
BAF_1-A:	Bagg	58	0	58	31	27(46.7%)
	Yale	58	0	58	44	14(24.1%)
BAF_1-B:	Bagg	50	3	47	18	29(61.7%)
	Yale	50	3	47	30	17(36.1%)
DIET 2						
ABF_1-A:	Bagg	51	4	47	19	28(59.6%)
	Yale	52	4	48	37	11(22.9%)
ABF_1-B:	Bagg	54	1	53	29	24(45.3%)
	Yale	54	0	54	42	12(22.2%)
BAF_1-A:	Bagg	50	0	50	30	20(40.0%)
	Yale	48	0	48	41	7(14.6%)
BAF_1-B:	Bagg	51	2	49	32	17(34.7%)
	Yale	56	4	52	33	19(36.5%)

* Anesthetic deaths, death within one week after irradiation.

and strands of cells. There are a moderate number of mitotic figures. In the gross it is a multiloculated tumor with a firm consistency and a uniform ivory-tan cut surface. It grows slowly, rarely becomes hemorrhagic, and has little tendency to form cysts. It is usually encapsulated and almost never metastasizes. At comparable ages it is smaller than the Yale tumor and grows more slowly.

The Yale tumor is a well vascularized, rather medullary, mammary carcinoma with few acini. The cells differ widely in their size, shape, and staining properties. Mitoses are frequent. It grows rapidly and early in its course becomes hemorrhagic, necrotic, and cystic. It usually erodes through the skin, in contrast to the Bagg tumor which seldom ulcerates. It generally has a thin capsule about most of the tumor and metastasizes infrequently to the regional lymph nodes. Microscopically and biologically it is much more malignant than the Bagg carcinoma.

In 7 of the 8 groups of experimental animals, approximately half the Bagg tumors were cured as against one-quarter of the Yale tumors. If the diets are pooled as being not significantly different, this two to one ratio holds throughout.

EFFECT OF FOSTER NURSING

The influence of foster nursing on the Yale tumor is not clear. Milk alone is apparently not enough to change the reaction of a tumor. In combination with other factors it may be. In those animals from strain A mothers foster nursing seemed to have had no effect. Thus 23.3 per cent of one group and 24.0 per cent of the other were cured, as can be seen in Table I. In the 106 animals derived from C57 black mothers, and foster nursed on strain A milk, 19.8 per cent were cured. These 3 percentages are not significantly different. On the other hand, 36.4 per cent of the mice from C57 black mothers, nursed on C57 black milk, were cured. This figure, although not significantly different from either 23.3 per cent or 24.0 per cent, is nevertheless of some significance when compared with the BAF_1-A figures. Additional work was designed to clarify these results and will be described later in this report.

Concerning the Bagg tumor there was no demonstrable effect of breast milk, either alone or in combination with parentage. This may be the case with the Yale tumor since the differences are only barely statistically significant (Tables I and IV).

HISTOLOGICAL RESPONSE

Control tumors.—There is no appreciable difference in the appearance of the tumors, or in the stroma reaction, which has any correlation with diet, breast milk, or parentage.

Radiated tumors.—In most of the tumors seen soon after radiation there is some increase in stroma response. This is similar to that observed by Oughterson, Tennant, and Lawrence (8) and consists of subdivision of the tumor by strands of fibrous tissue with an increase in the more minute filaments of connective tissue.

In those tumors which have clinically been temporarily inhibited by irradiation and have then taken on renewed growth the appearance is similar to that of untreated tumors.

For a complete study of the stroma response pathological sections would have to be taken at intervals between irradiation and death. This was not the concern of our investigation. However, from the available material and from the clinical results, the tumors seem to respond exactly as do those in pure strain A and in F_2 hybrids.

DISCUSSION

Had only one tumor been inoculated into each mouse, the difference in radiosensitivity would possibly have been more pronounced. Because it is a faster growing tumor, the Yale carcinoma usually outstripped the Bagg and probably accounted for most of the deaths. In the course of 4 months, several tumors which had regressed following irradiation eventually reappeared. Thus when an animal died early with an apparent cure of one tumor, that tumor might have reappeared in a few weeks. However, when a regressing tumor ultimately disappeared toward the close of the 4 month interval, it rarely came back. Since most of the early deaths were due to rapidly growing Yale tumors, it is possible that many regressing Bagg tumors were destined for cure but were not counted as such. This, and the possible effect of one neoplasm upon the other, are the chief objections to using a single mouse for more than one tumor.

There is little question that the response of the tumors to x-ray was unaffected by dietary differences. It may be that delicate biological effects would be obscured by our test. A smaller radiation dosage might be a more sensitive detector of dissimilarities. It is debatable whether one should repeat the work with this in mind.

Foster nursing did not influence the response of the Bagg tumor. The results with the Yale carcinoma are not so clear. There was one group in which the cure rate was significantly higher than the others. This was the set of mice with C57 black mothers, nursed on C57 black milk. The milk alone was not enough to influence the response to radiation. As can be seen from Table I, the ABF_1-A and ABF_1-B mice behaved exactly alike, despite the difference in the milk. Nor can one ascribe a dominating influence to a factor which may have been inherited from the black mother, for the ABF_1-B and BAF_1-B mice produced statistically the same cures. The combination of C57 black mothers and black milk may be effective, but it is difficult to discover the mechanism of this possible influence. The curability is certainly not controlled by a single dominant mendelian character. If this were so, then all the F_1 hybrids should have behaved alike. To argue for genetically controlled influences, one would have to grant that multiple factors were involved. Because the genes are not segregated in the F_1 generation, there are insufficient data for further discussion along possible genetic lines.

TABLE IV: INFLUENCE OF MILK FACTOR
TREATED ANIMALS
Yale Tumor Radiated with 2,500 r

	Number treated	Dead with tumor	Dead, tumor cured	Cured, per cent	Not cured, per cent
AYF_1-A	48	47	1	2.1	97.9
AYF_1-Y	24	24	0	0.0	100.0
YAF_1-A	50	49	1	2.0	98.0
YAF_1-Y	48	46	2	4.2	95.8

TABLE V: INFLUENCE OF MILK FACTOR
CONTROL REGRESSIONS
No Radiation; Yale Tumor

	Regressed	Dead with tumor	Total	Regressions, per cent
AYF_1-A	0	26	26	0
AYF_1-Y	0	14	14	0
YAF_1-A	0	24	24	0
YAF_1-Y	0	21	21	0

In order to clear up these inconclusive results another set of experiments was designed. Pure Y strain mice were crossed with those of strain A. Some of the first generation litter were foster nursed and the remainder were not. Into each of the F_1 animals was inoculated the Yale mammary carcinoma. Only one tumor was transplanted into each animal. This was radiated after a suitable time with 2,500 r with the factors cited previously. All the tumors took. Sufficient controls were observed in each group. The controls were exactly like the test animals except that they received no radiation. The results are shown in Tables IV and V. Because of a peculiar reluctance of Y mothers to nurse any but their own litters (a characteristic observed by us in no other mice) one group has fewer animals than the others. However, we believe the results to be significant. We think they indicate conclusively that the milk factor has no influence on the response to x-ray of a transplanted mammary tumor. The ambiguity of the previous results must have been due to some factor other than the

breast milk. Since in the first series of experiments (but not the later ones) each mouse was inoculated with 2 tumors, the equivocal results were possibly due to the biologic effect of one tumor upon the other as both grew simultaneously in the same animal.

In 1926 Strong (11) demonstrated that transplanted tumors in heterozygous mice grew faster than they did in homozygous strains. When one considers that previous work has shown that strain A mice, inoculated with the Yale tumor and then radiated with a single dose of 2,500 r, showed a 1 per cent cure, and that F_2 hybrids similarly treated produced an 82 per cent cure, it appears that our own F_1 generation cures of 19 to 36 per cent represent an intermediary stage between the others. In other words, the cure rate increased hand-in-hand with both the heterozygosity and the degree of biological variation in the host.

Transplantability is a distinct and separate property, unrelated to radiosensitivity. A single dominant genetically inherited character seems to influence the transplantability of the Yale tumor. This neoplasm takes in 100 per cent of animals in the F_1 generation. In the F_2 hybrids 75 per cent of the tumors will take. In other words, this is a 3 to 1 ratio, characteristic of a single mendelian factor. The mechanism behind radiosensitivity, as has been shown, is decidedly more complex.

SUMMARY

The effect of diet and foster nursing on 2 separate transplanted mouse tumors was investigated. The tumors used were the Yale mammary carcinoma and the Bagg-Jacksen tumor. The mice which were inoculated were F_1 hybrids obtained by crossing strain A albinos and C57 blacks, and others obtained by crossing the Y and A strains. One-half the hybrids were foster nursed. Radiation was used as an index of difference in behavior of the tumors in these hosts.

Despite the fact that their hosts (and the parents of the hosts) had been fed different diets, all the examples of each of the tumors behaved alike.

The natural characteristics and behavior of the Bagg-Jacksen and Yale tumors are dissimilar. The Yale tumor is the more malignant, its proportion of cures being just half that of the Bagg-Jacksen.

Foster nursing alone has no influence on the radiosensitivity of transplanted Bagg or Yale mammary tumors.

The results of this experiment are complicated by a fault in technic. In one group of experiments, 2 tumors were inoculated into each animal, and the growth characteristics of one tended to obscure those of the other. The results were clarified when only one tumor was employed.

The authors are indebted to Dr. John J. Bittner and Dr. L. C. Strong for advice and criticism, and to Dr. H. J. Bagg for the Bagg-Jacksen tumor.

Appreciation must be expressed for the invaluable technical assistance of Mr. Leonard Smith and Mr. Vincent Tucker.

REFERENCES

1. Bagg, H. J., and Jacksen, J. The Value of a "Functional Test" in Selecting Material for a Genetic Study of Mammary Tumors in Mice and Rats. Am. J. Cancer, **30**:539-548. 1937.
2. Bittner, J. J. Differences Observed in an Inbred Albino Strain of Mice Following a Change in Diet. Nutritional Bull., Roscoe B. Jackson Mem. Lab., **1, 2**. 1936.
3. Bittner, J. J. The Breeding Behavior and Tumor Incidence of an Inbred Albino Strain of Mice. Am. J. Cancer, **25**:113-121. 1935.
4. Bittner, J. J. Differences Observed in the Tumor Incidence of an Albino Strain of Mice Following a Change in Diet. Am. J. Cancer, **25**:791-796. 1935.
5. Bittner, J. J. Breast Cancer in Breeding and Virgin "A" and "B" Stock Female Mice and Their Hybrids. Pub. Health Rep., **54**:1113-1118. 1939.
6. Cramer, W. The Therapeutic Action of Radium on Spontaneous Mammary Carcinomata of the Mouse. Scient. Rep. Invest. Imp. Cancer Research Fund, **11**:127-146. 1934.
7. Gardner, W. U., Smith, G. M., Allen, E., and Strong, L. C. Cancer of the Mammary Glands Induced in Male Mice Receiving Estrogenic Hormone. Arch. Path., **21**: 265-272. 1936.
8. Oughterson, A. W., Tennant, R., and Lawrence, E. A. The Tumor Response and Stroma Reaction Following X-Ray of a Transplantable Tumor in Inbred Strains of Mice. Yale J. Biol. & Med., **12**:419-425. 1940.
9. Strong, L. C. The Incidence of Spontaneous Tumors of Mice of the CBA Strain after a Change of Diet. Am. J. Cancer, **32**:80-84. 1938.
10. Strong, L. C. The Incidence of Spontaneous Tumors on a "Mixed Oatmeal Diet." Gann, **31**:13-16. 1937.
11. Strong, L. C. A Genetic Study of the Growth of a Transplantable Tumor (Adenocarcinoma dBrB). J. Exp. Zool., **45**:231-253. 1926.

Morphological Aspects of Experimental Actinic and Arsenic Carcinomas in the Skin of Rats

W. C. Hueper, M.D.

(From the Warner Institute for Therapeutic Research, New York, N. Y.)

(Received for publication April 25, 1942)

Epidermal neoplasms of the human skin vary greatly in their degree of differentiation and in the type of special organic morphology which they reproduce. However, this diversity in structure is apparently unrelated to the physical or chemical nature of the causative carcinogenic agent. Thus, epidermoid carcinomas as well as basal cell cancers are elicited by the action of arsenicals and solar radiation containing ultraviolet rays. Similar conditions seem to apply to animals. Putschar and Holtz (8) observed in the skin of rats exposed to ultraviolet rays not only these 2 types of blastomas, but also trichoepitheliomas and spindle cell sarcomatoid carcinomas resembling those found not infrequently in the skin of mice subjected to paintings with tar. These observations show that the skin of rats possesses blastomatous potentialities which are equal to those of the human skin in spite of the fact that these tissues differ normally in their anatomical structure to a considerable degree.

The following study on the morphological aspects of experimental actinic and arsenic carcinomas in the skin of rats is presented to emphasize the structural diversity of the various epithelial tumors produced by these carcinogenic agents and to add at the same time several new types of epidermal neoplasms hitherto not described in irradiated rats.

ACTINIC CARCINOMAS

MATERIAL

The observations to be reported were made in a series of 20 rats which had been exposed, for a period of up to 10 months, to the radiation emitted from the mercury vapor burner of a Hanovia Super S Alpine Lamp yielding 1,500 milliwatts per square centimeter at a distance of 75 cm. Details concerning the experimental procedures which led in these animals to the development of cancers of the skin, as well as brief notes regarding the types of tumors produced, have been given in a previous paper (3).

HISTOLOGICAL DATA

Epidermal Hyperplastic Reactions

The primary effect of an exposure to ultraviolet rays is a thickening of the cornified lamellated layer and an increase in the number of cellular layers from the normal 1 or 2 to 5 or 6. Simultaneously, there occurs a progressive differentiation of the epidermal cells in the more superficial layers. They change from relatively small oval or round cells to large polygonal cells which, near the cornified lamella, contain eleidin granules. The cells of the basal layer not infrequently assume a low cuboidal shape and become arranged in regular palisade formation. The basal layer is covered in some areas by one or several layers of somewhat larger round cells.

While these changes represent the most frequent epidermal reaction, there occur regions in which a diffuse and irregular proliferation of basal and round cells takes place without any appreciable thickening of the cornified layer and any differentiation into squamous cells (Fig. 1). Some of these atypical epidermal areas are covered by a layer of debris and leucocytes. They vary considerably in thickness and may include areas composed of spindle cells and squamous cells (Fig. 2).

Following or accompanying these manifestations of epidermal proliferation, differentiation, and disorganization, small well circumscribed buds of round and oval cells project into the subepidermal vascular connective tissue. These processes may preserve during further growth their oval, round, or spindle cell character, or they may develop in their central portions occasional small epithelial pearls surrounded by a few flattened squamous cells, or they may undergo progressive differentiation in these parts with the formation of large masses of polygonal cells and numerous pearls. The course last mentioned is the most frequent one, and is often associated with the development of solid epithelial papillary projections covered by a thick cornified layer and extending beyond the surface of the skin.

The increased keratinization of the epidermis results

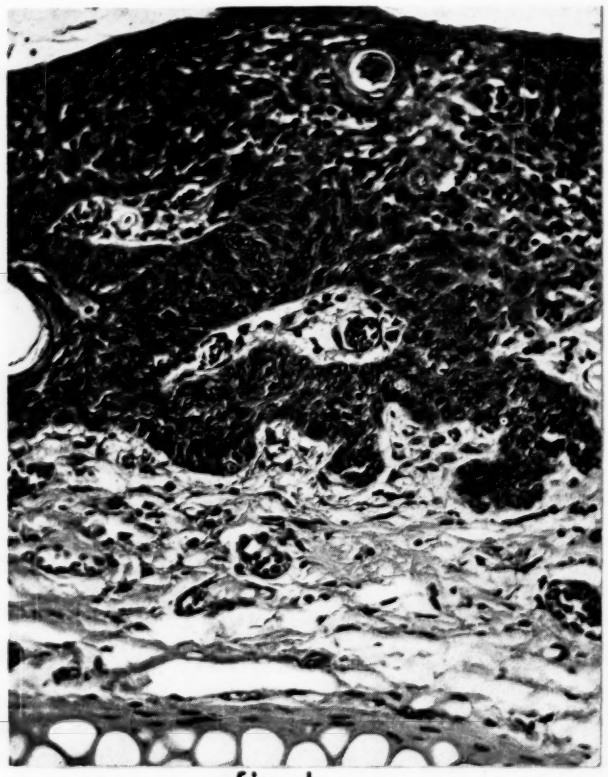

fig. 1

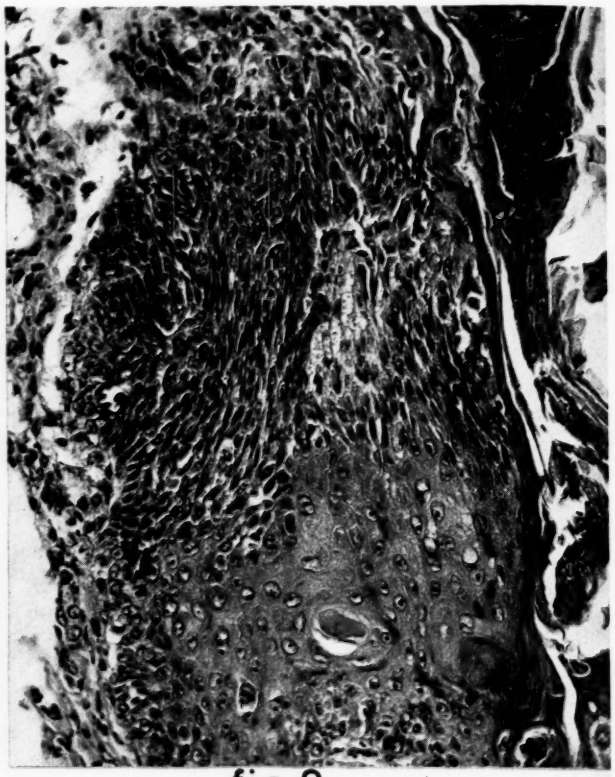

fig. 2

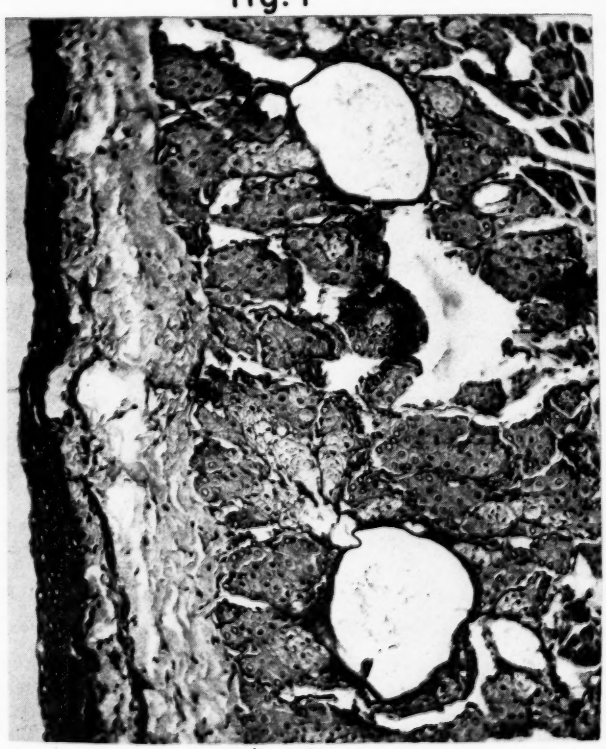

fig. 3

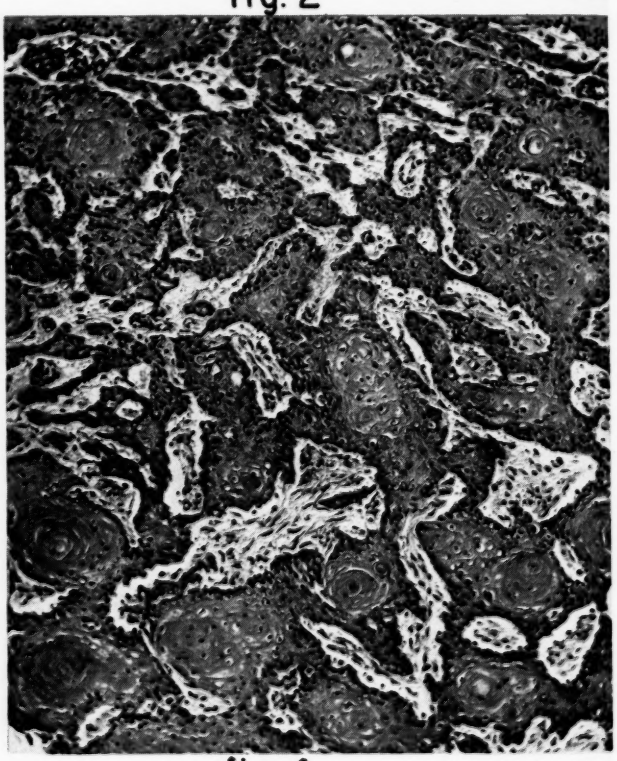

fig. 4

FIG. 1.—The epidermis shows proliferation of round cells, which extend in garland formations into the deeper tissue. Mag. × 155.

FIG. 2.—Beneath a thick cornified layer lies a thickened epidermis, consisting of areas of densely packed spindle epithelial cells and squamous cell parakeratotic foci. Mag. × 155.

FIG. 3.—Underneath a hyperplastic epidermis large accumulations of sebaceous glands are found. Mag. × 75.

FIG. 4.—Squamous cell carcinoma with cornification. Mag. × 75.

not infrequently in a clogging of the orifices of the hair follicles which subsequently become distended thereby and filled with keratinized material and fragments of degenerated hair. The sebaceous glands occasionally undergo notable proliferation, and then form dense adenomatoid clusters (Fig. 3).

These hyperplastic epidermal reactions form the basis for the subsequent development of various types of malignant tumors, whose morphological features are sometimes influenced to an appreciable degree by the benign or malignant proliferative processes affecting concomitantly the underlying mesenchymatous tissues.

Epidermal Neoplastic Reactions

(a) The most common type of actinic carcinoma in the skin of rats subjected to ultraviolet radiation is the squamous cell carcinoma with cornification (Fig. 4). It is identical in all morphological respects to that seen in the human skin.

(b) The squamous cell carcinoma without cornification is considerably less often encountered under the experimental conditions stated (Fig. 5).

(c) The third type of epidermal neoplasm not infrequently met with in the skin of irradiated rats is a round cell carcinoma consisting of densely and irregularly packed, relatively large, not always well defined, round cells, which grow in large plump processes and sheets in which a marginal germinal layer is not discernible (Fig. 6).

(d) In some instances the cells of such an immature neoplasm have an oval or spindle shape, either in scattered areas or throughout the bulk of the tumor. These spindle cell carcinomas differ, however, from the ordinary basal cell carcinoma of the human skin in that their cells are larger, they have a more abundant amount of cytoplasm, and the cellular outlines are sometimes more distinct than those seen in the spindle cell type of basal cell cancer of human origin. Morphologically they resemble more closely the spindle cell carcinomas found in the human uterine cervix (Fig. 7). Inasmuch as spindle cell sarcomas are not rare in the cutaneous tissue of irradiated rats (Fig. 8) and occur in coexistence with epidermal neoplasms, it is occasionally difficult to distinguish between these 2 types of tumors, especially when they collide in the subcutaneous tissue. The use of silvered preparations for the demonstration of reticulum and the preparation of sections stained with phosphotungstic acid-hematoxylin for the demonstration of fibroglia and myoglia is of aid in the differential diagnosis of these tumors (2).

(e) Carcinomas of the basal cell type, consisting of relatively small, round, oval, or spindle cells with ill defined outlines and hypochromatic nuclei growing either in large, well demarcated islands (Fig. 9) or composed of hyperchromatic cells forming slender, garland-like, reticulated structures in a loose, partly mucinous stroma (cylindromatous type) (Fig. 10) occur rarely.

(f) Somewhat more frequent are combinations of basal cell cancer and squamous cell carcinoma (keratinizing basal cell carcinoma, Fig. 11). The ratio of the 2 types of structures varies greatly with the individual tumors.

(g) A very rare variety, which was seen only once in the present series, is represented by an adamantinomatoid type of carcinoma. This is composed of smaller or larger nests of well outlined cells of spindle shape surrounded by columnar elements resembling enameloblasts. In some of these alveoli pinkish stained pearls are present, which are embedded in a reticular, cellular matrix. The intervening stroma is very loose and vascular (Fig. 12).

(h) A pseudoglandular type of carcinoma occurs infrequently. It originates from a solid type of epithelial growth consisting of medium sized, polygonal, or cuboidal elements which undergo degenerative and lytic changes in the central portion of the strands and islands, leaving the peripheral cell layers intact. This process results in the formation of tubular or alveolar structures lined by somewhat irregularly arranged epithelial cells (Fig. 13), such as are seen in pseudoglandular carcinomas of the bladder in dogs treated with β-naphthylamine (3).

This tumor displays a definite similarity to angiosarcomas occurring in the subcutaneous tissue of irradiated rats and bordering on or involving the epidermis. It is important that in the case of angiosarcomas the overlying or adjacent epidermis displays merely hyperplastic changes without any appreciable atypical manifestations (Fig. 14).

(i) The blastomatogenic effect exerted by ultraviolet rays on both the epidermal and vascular elements of the skin is exemplified, moreover, by the occurrence of squamous cell carcinomas possessing a loose, edematous, stroma which contains numerous dilated and congested blood vessels (Fig. 15). Such tumors have been termed angio-epitheliomas when occurring in the human bladder or in the skin of mice painted with tar.

(j) A demonstration of the carcinogenic effect of ultraviolet rays upon epithelial structures situated in the subepidermal tissues is presented by the occasional occurrence of carcinomas reproducing in a defective form the structure of the sebaceous glands (sebaceous carcinomas, Fig. 16). These tumors consist of large, well circumscribed alveoli filled with distinctly outlined epithelial cells possessing a foamy cytoplasm.

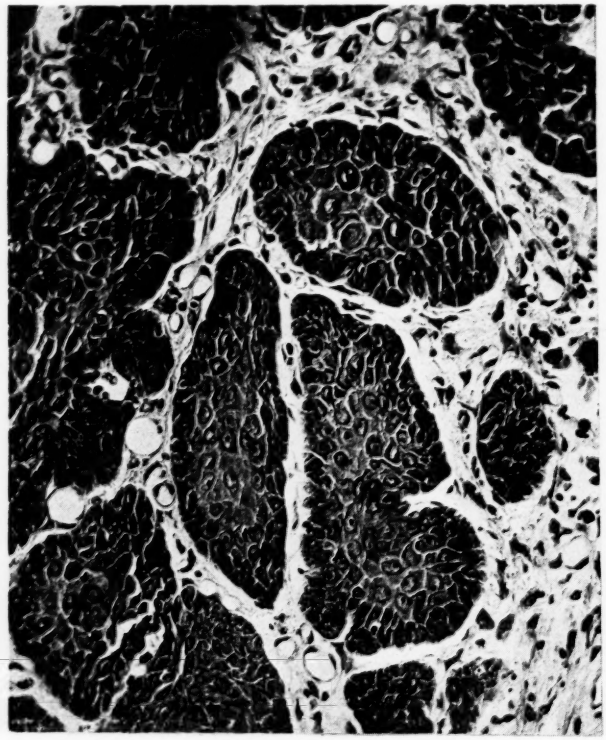

fig. 5

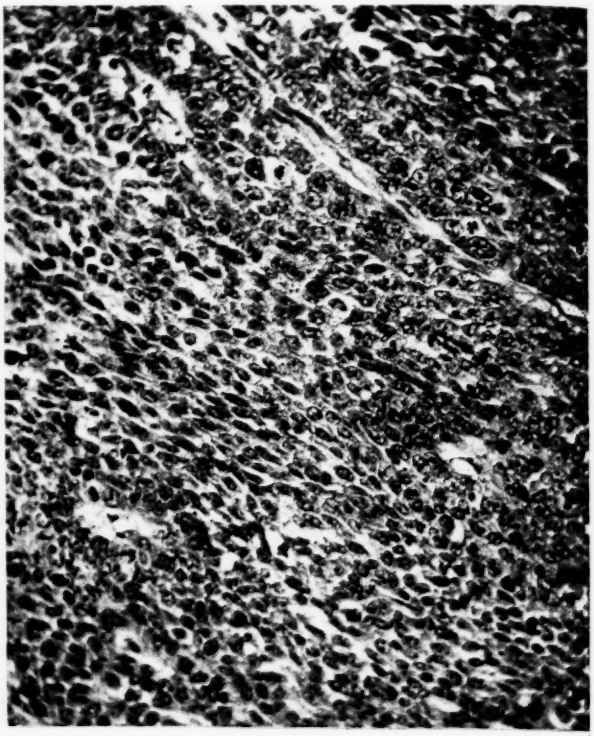

fig. 6

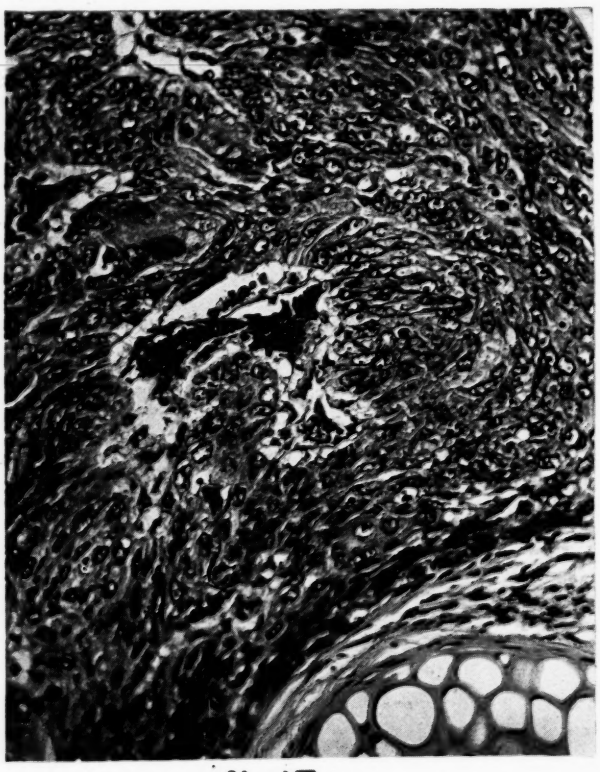

fig. 7

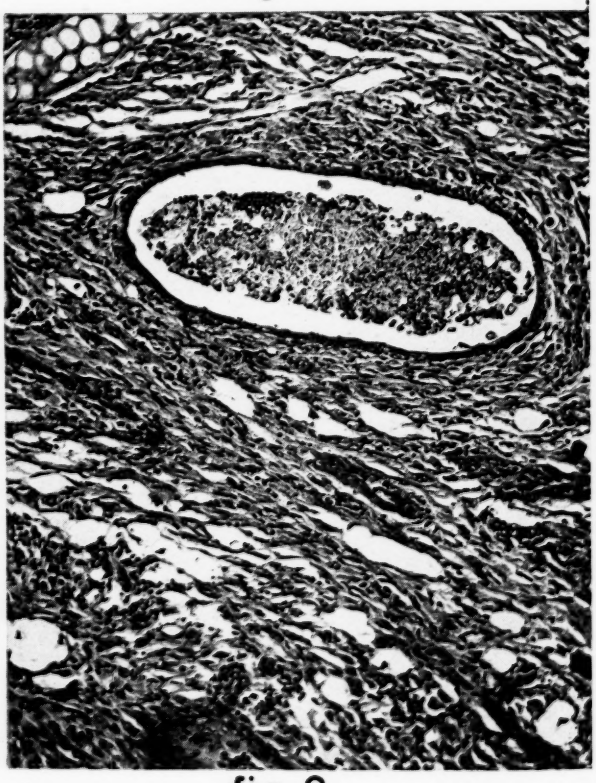

fig. 8

FIG. 5.—Squamous cell carcinoma without cornification. Mag. × 155.
FIG. 6.—Round cell carcinoma with densely packed indistinctly outlined round and oval cells. Mag. × 155.
FIG. 7.—Epidermal carcinoma consisting of oval and spindle shaped cells. Mag. × 155.
FIG. 8.—Spindle cell sarcoma surrounding an epidermal cell nest with central necrosis. Mag. × 75.

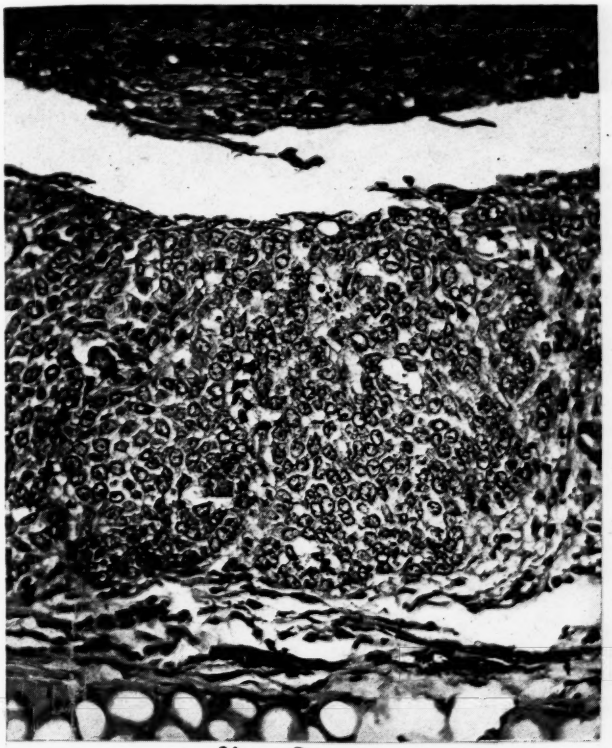

fig. 9

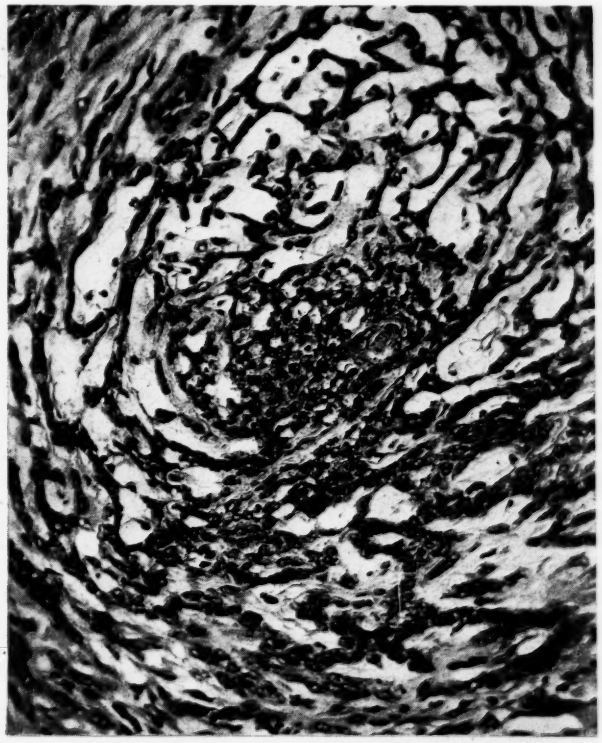

fig. 10

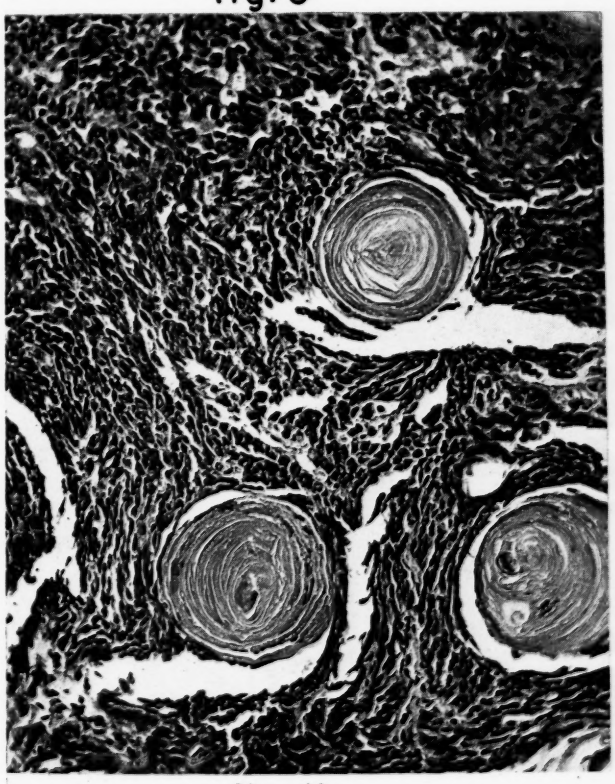

fig. 11

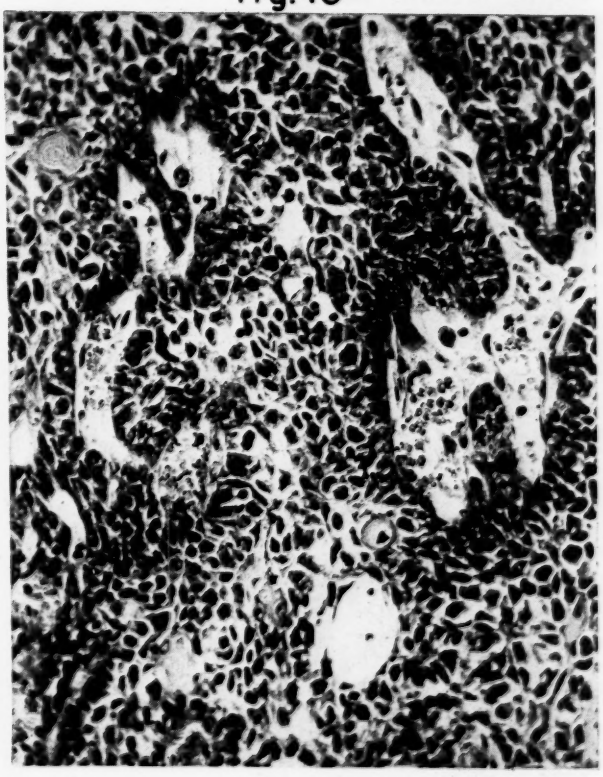

fig. 12

FIG. 9.—Small, ill defined round cells with superficial necrosis advancing toward the auricular cartilage. Mag. × 155.

FIG. 10.—Small round and spindle cells growing in reticular formation with a scanty loose stroma. Mag. × 155.

FIG. 11.—Densely packed hyperchromatic round and spindle cells with numerous epithelial pearls. Mag. × 155.

FIG. 12.—Irregularly arranged cords of epithelial cells lined by low cuboidal cells which surround a reticulate cellular mass. The stroma is scanty and very vascular. Mag. × 155.

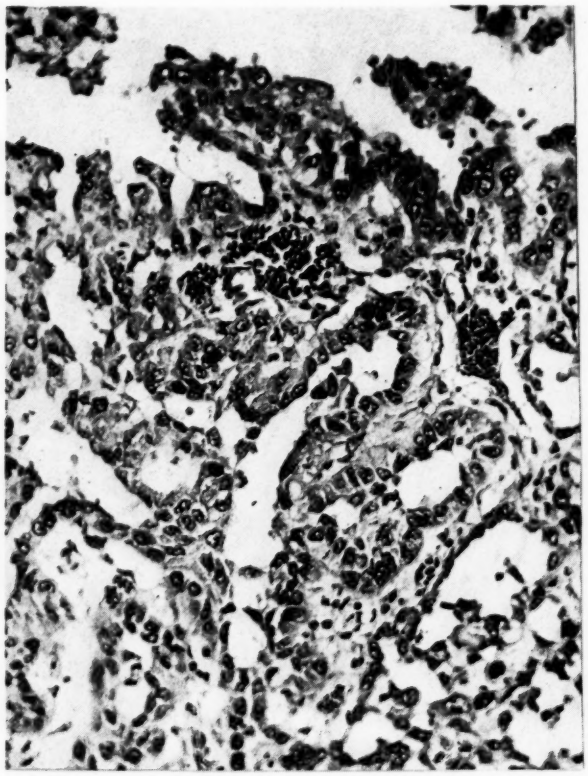

fig. 13

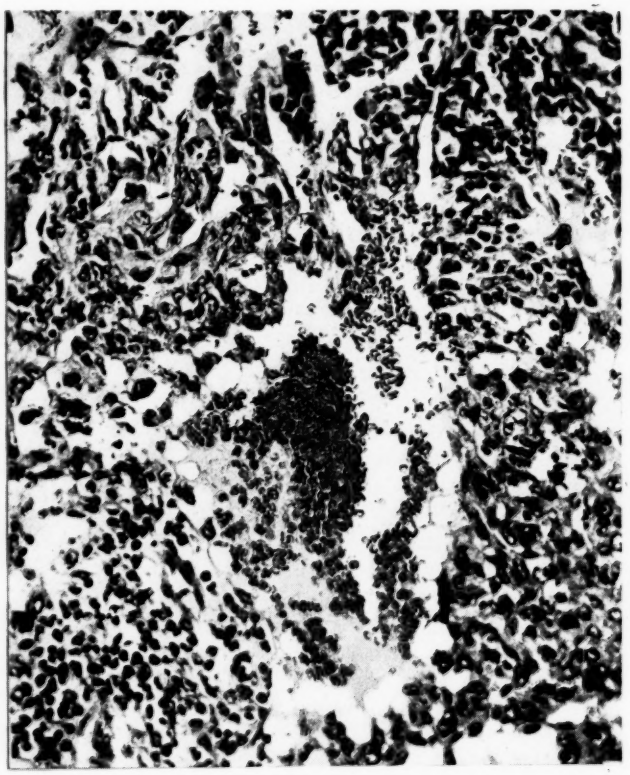

fig. 14

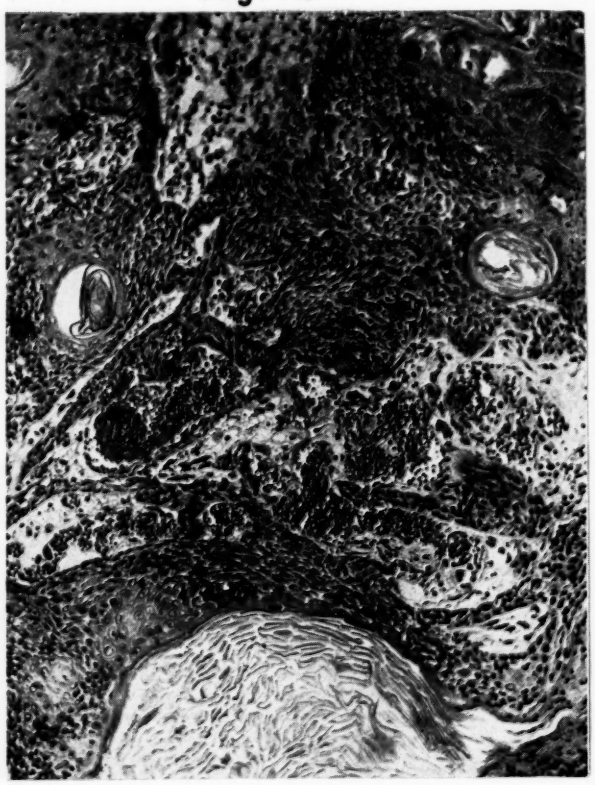

fig. 15

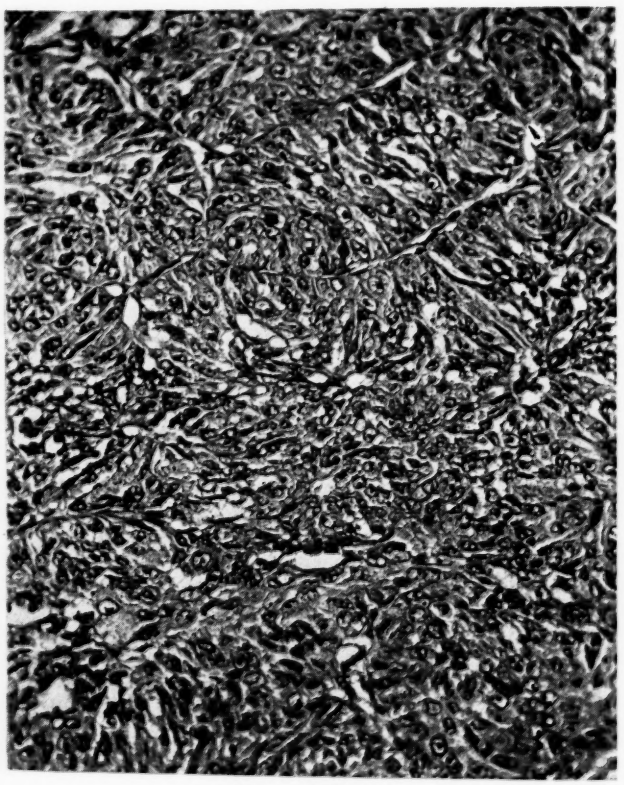

fig. 16

FIG. 13.—Tubular and alveolar cavities lined by irregularly arranged cuboidal cells representing pseudoglandular carcinoma. Mag. × 155.

FIG. 14.—Ill defined cells of irregular shape and size lining small and large cavities filled with erythrocytes and forming in part the cellular framework of these structures. Mag. × 155.

FIG. 15.—Atypical squamous cell carcinoma with an extraordinary development of vascular stroma. Mag. × 75.

FIG. 16.—Densely packed long, oval, and spindle epithelial cells with a foamy cytoplasm arranged in relatively well defined alveoli showing a certain resemblance to sebaceous glandular structures. Mag. × 155.

(k) A relatively rare type of actinic carcinoma is represented by anaplastic carcinomas. They are characterized by a kind of dissolution of the epidermis into highly atypical cells which vary greatly in size, shape, staining properties, and distinctness of outline. They invade the subcutaneous connective tissue diffusely in small groups or as solid sheets (Fig. 17). Their diagnosis sometimes offers great difficulties, as they have to be distinguished from equally atypical, polymorphous cell sarcomas occurring in the subcutaneous tissue of irradiated rats (Fig. 18) and in direct contact with the often hyperplastic and hyperkeratotic epidermis. This usually shows an intact basal layer.

(l) The relatively thin epidermis of the rat, which permits a ready penetration of the ultraviolet rays into the subepidermal connective tissue, is responsible not only for the frequent occurrence of sarcomas in these tissues but also for that of carcinosarcomatous collision tumors. They are usually squamous cell carcinomas with an atypical, spindle cell sarcomatous stroma (Fig. 19).

ARSENIC CARCINOMAS

In spite of the fact that the epithelium lining the clogged and distended hair follicles was usually hyperplastic and hyperkeratotic, there was not a single instance of trichoepithelioma found in the ultraviolet light series. This is remarkable, as these tumors are relatively common in the skin of mice subjected to applications of tar, which penetrates into the lumen of the follicles and thus establishes direct contact with their epithelial lining. It is of significance for this reason that a tumor showing trichoepitheliomatous structure was observed in 1 out of 10 congenitally hairless rats which had received with their drinking water increasing amounts of arsenious acid in the form of Fowler's solution (Fig. 20). Hairless rats were employed in this experiment because the absence of hair impairs the ready excretion of arsenic by the skin and its appendages and thus favors the retention of this carcinogenic agent, especially in the epithelial lining of the cystically distended hair follicles. Hairless rats normally develop papillary warts in their hyperkeratotic skin, which possibly might possess an increased tendency toward a malignant degeneration under the stimulus of an exogenous carcinogenic agent.

The tumor was found in the last rat surviving for 21 months. It was located beneath the epidermis in the subcutaneous tissue and represented a more or less well defined nodule measuring about 1 cm. in diameter. It was the only tumor observed in this series as well as in a series of haired and identically treated control rats which were litter mates of the hairless rats. Six of these haired rats survived for more than 23 months without showing any skin lesions.

DISCUSSION

The evidence presented is satisfactory proof that under proper stimulation (ultraviolet rays, arsenic) the epidermis of the rat is capable of producing as great a variety of carcinomas as that seen in the human skin. The carcinomas obtained in the skin of rats following an exposure to ultraviolet rays are even more complex than those occurring in man, as they are not infrequently associated with hyperplastic or sarcomatous proliferations of the mesenchymatous elements of the cutis, complications rare in tumors of the human skin. The great variety of epithelial tumors and their frequent combination or coexistence with mesenchymatous neoplasms observed in these animals is attributable to some extent to the fact that the skin of the rat is much thinner and less cornified than the human skin, so that the ultraviolet rays can penetrate deeper into the subepidermal tissue and elicit blastomatous responses both from the appendages of the skin and from the mesenchymatous tissues.

However, the occurrence of tumors differing widely in structure, yet located side by side in the skin of the exposed animals, indicates that the diversity depends not entirely upon the action of the carcinogenic agent as determined and modified in its degree and depth by the thickness of the keratin and cellular layer and by the density and distribution of pigmentation and hairs. The type of photochemical changes elicited in the skin seems to play a major role in this respect. These, in turn, seem to depend upon endogenous factors of metabolic nature and are responsible for the kind of pathological "internal environment" produced in the irradiated tissue, thereby providing a certain steering mechanism in the carcinogenic process.

The validity of this concept receives some support from the fact that repeated attempts to elicit carcinomas of the skin in rabbits by prolonged exposure to ultraviolet rays have failed consistently, in spite of the production of such tumors with the aid of another actinic agent, roentgen rays. Inasmuch as the anatomical structure of the skin of the rabbit does not differ essentially from that of the rat it has been argued that intrinsic factors related to the herbivorous metabolism of the rabbit, and particularly to its lipoidal aspects, are responsible for this resistance of the skin.

Baumann and his associates (1) attempted, therefore, to overcome this metabolic influence by feeding cholesterol to rabbits during the course of irradiation treatment. However, their results were negative. The writer's own experiments, conducted for a similar

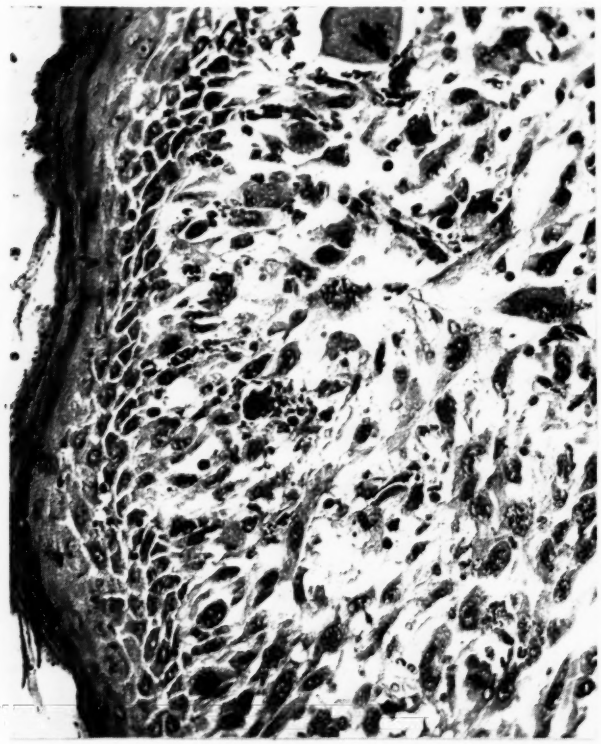

fig. 17

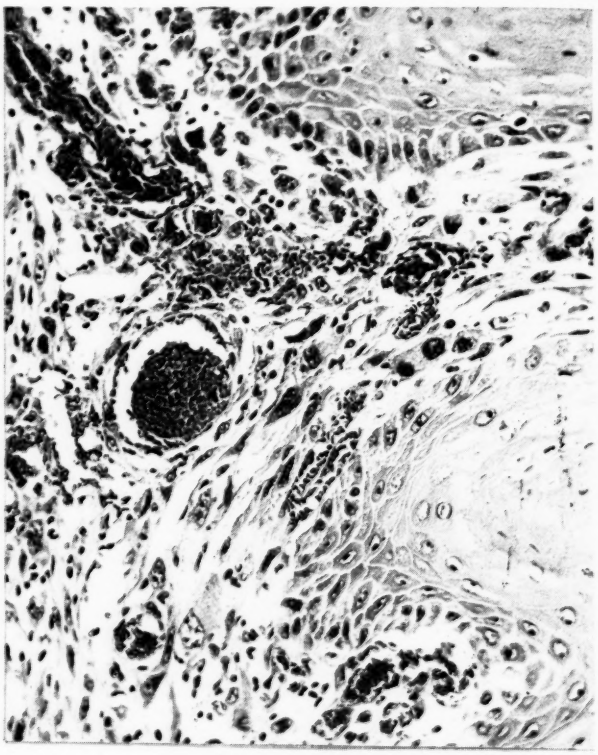

fig. 18

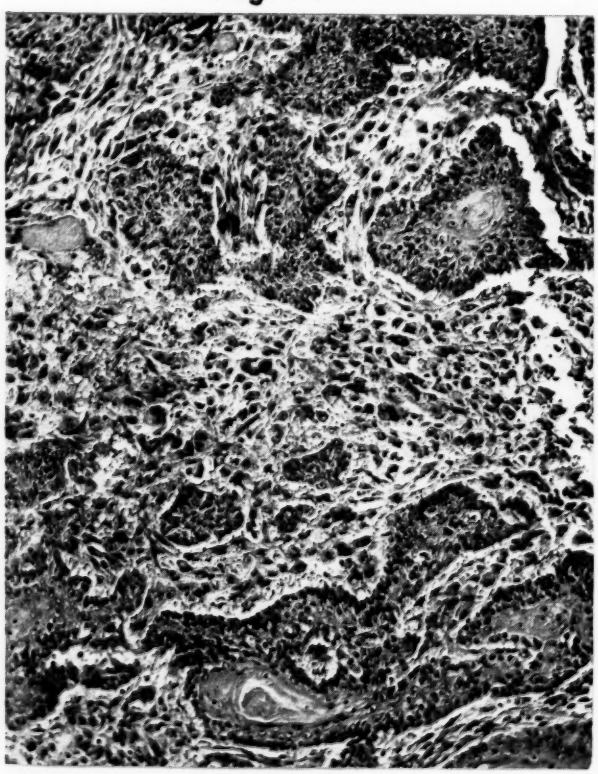

fig. 19

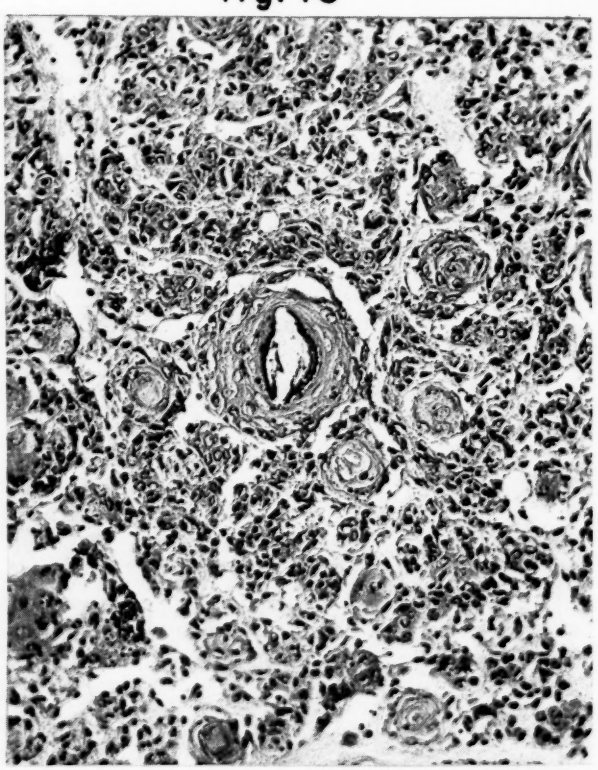

fig. 20

FIG. 17.—Diffuse and highly anaplastic proliferation of the basal cell layer into the subepidermal tissue. Mag. × 155.

FIG. 18.—Polymorphous cell sarcoma underneath a hyperkeratotic and hyperplastic epidermis. Mag. × 155.

FIG. 19.—Nests of cornified squamous cell carcinoma imbedded in a polymorphous cellular sarcoma. Mag. × 75.

FIG. 20.—Densely packed nests of small cuboidal and oval cells surrounding frequently hyaline lamellated foci which contain occasional abortive hair formations. Mag. × 155.

purpose several years ago with 17 white coated and blue eyed rabbits (crosses between Chinchilla and New Zealand Red rabbits), were equally unsuccessful. The skin of the back in 8 of these rabbits was shaved with an electric razor daily and was then exposed for increasing periods of time to ultraviolet rays. Moreover, the rabbits were given Fowler's solution by mouth in order to provide an additional specific carcinogenic stimulus and to enhance at the same time the photochemical action of the rays by the production of a chemical photosensitivity. A control series of 9 rabbits received actinic treatment only. During the experimental period, which lasted up to 12 months, all rabbits developed a chronic dermatitis with multiple and sometimes deep ulcerations, keratoses, pigmentary shifts, disturbances in the growth of hair, scaling, chronic congestion, etc. The epidermis of the back and of the ears showed hyperplastic, hyperkeratotic, and atrophic changes. These alterations were not infrequently associated with numerous small ulcerations and with a ballooning of the basal cells. The basal cell layer of the skin of the ears often exhibited a diffuse proliferative budding of these cells, but in only one instance was there a displacement of discrete basal cell nests into the deeper tissue. The definite proliferation of the auricular cartilaginous tissue occasionally noted provided additional evidence concerning the deep penetration of the rays. These cutaneous changes were most decided in the rabbits of the arsenic series. Thus, endogenous metabolic factors seem to control the type and degree of proliferative epithelial response to ultraviolet rays, and in some species apparently exert a sort of protective action which, however, may not be absolute, as shown by similar observations made in connection with tar carcinogenesis.

Although arsenical epidermal cancer in man has appeared in the form of cancroid, mixed basal and squamous cell carcinoma, basal cell cancer, and Bowen's dyskeratosis, the few experimental arsenic carcinomas produced in mice or rabbits have all been squamous cell carcinomas (4, 9). The trichoepithelioma found in the skin of a congenitally hairless rat treated with Fowler's solution represents, therefore, a new morphological type of epidermal cancerous response to this agent. The frequent failure experienced in attempts to cause cancer of the skin in animals (mice, rats, rabbits) by the oral, parenteral, or cutaneous administration of various arsenicals may be related to the fact that arsenic cancer has a relatively long latent period, usually surpassing that of the average life span of the species used in such experiments. This contention is supported by the observation that animals with a much longer life span (horses, sheep, deer) have developed an environmental type of arsenic carcinoma of the skin or of the nasal mucous membranes (5-7).

CONCLUSIONS

The evidence presented is proof that the epidermis of the rat is capable of producing under proper stimulation (ultraviolet rays, arsenic) as great a variety of carcinomas as that seen in the human skin.

The carcinomas obtained in the skin of the rat following an exposure to ultraviolet rays are even more complex than those occurring in man, as they are not infrequently associated with hyperplastic or sarcomatous proliferations of the mesenchymatous elements of the cutis.

The average life span of the rat is apparently shorter in general than the latent period of experimental arsenic carcinoma.

REFERENCES

1. Baumann, C. A., Rusch, H. P., Kline, B. E., and Jacobi, H. P. Does Cholesterol Stimulate Tumor Development? Am. J. Cancer, **38**:76-80. 1940.
2. Grady, H. G., Blum, H. F., and Kirby-Smith, J. S. Pathology of Tumors of the External Ear in Mice Induced by Ultraviolet Radiation. J. Nat. Cancer Inst., **2**:269-276. 1941.
3. Hueper, W. C. Cutaneous Neoplastic Responses Elicited by Ultraviolet Rays in Hairless Rats and in Their Haired Litter Mates. Cancer Research, **1**:402-406. 1941. Occupational Tumors and Allied Diseases. P. 896. Springfield, Ill.: Charles C. Thomas. 1942.
4. Leitch, A. The Experimental Inquiry into the Causes of Cancer. Brit. M. J., **2**:1-7. 1923.
5. Nieberle, K. Über endemischen Krebs im Siebbein von Schafen. Ztschr. f. Krebsforsch., **49**:137-141. 1939.
6. Paris, J. A. Pharmacology. Third edition. P. 123. London: W. Philipps. 1822.
7. Prell, H. Die Schädigung der Tierwelt durch die Fernwirkungen von Industrieabgasen. Arch. f. Gewerbepath., **7**: 656-670. 1937.
8. Putschar, W., and Holtz, F. Erzeugung von Hautkrebsen bei Ratten durch langdauernde Ultraviolettbestrahlung. Ztschr. f. Krebsforsch., **33**:219-260. 1930. Erzeugung von Sarkomen und sarkomähnlichen Geschwülsten durch langdauernde Ultraviolettbestrahlung. Libro de Oro en homenaje al Prof. Dr. Angel H. Roffo. 1935.
9. Raposo, L. S. Le cancer à l'arsenic. Compt. rend. Soc. de biol., **98**:86. 1928. Sur le rôle de l'arsenic dans le cancérisation par le goudron. Compt. rend. Soc. de biol., **98**:997. 1928.

Factors Affecting Carcinogenesis

I. The Effect of Lipoid Solvents on Tumor Production by 3,4-Benzpyrene

Frank Dickens, Ph.D., and Hans Weil-Malherbe, M.D.

(From the Cancer Research Laboratory, North of England Council of the British Empire Cancer Campaign, Royal Victoria Infirmary, Newcastle-upon-Tyne, England)

(Received for publication April 17, 1942)

It is now well recognized that tumor incidence following the injection of carcinogenic hydrocarbons is greatly dependent upon the nature of the solvent used, both the percentage of tumors and the latent time of their appearance being affected. For convenience, we may distinguish between solvents which inhibit carcinogenesis and those which do not, although there is by no means a clear-cut separation. Moreover the same solvent may behave differently under different workers, for reasons as yet mostly undefined.

To those solvents which lead to a high incidence of tumors, even when relatively small doses of carcinogen are used, belong sesame oil (19), arachis oil (2), olive oil (13), paraffin (5), several synthetic glycerides (17), benzene (9), lard (1), and probably cholesterol, provided that the concentration of carcinogen is adequate (15, 16). Many of these solvents are mixtures of variable composition, and in the case of lard (17) their effect on carcinogenesis has been shown to vary from batch to batch; such differences have not yet been correlated with chemical composition, except that rancidity appears not to be an important factor (17).

On the other hand, tumor production may be prevented, diminished, or delayed when certain other solvents are employed. Watson (20) found that pinene tar mixed with rat tissue extract gave rise to no tumors on subcutaneous injection into rats, but mixed with paraffin wax it produced sarcoma in 3 of 12 rats. Peacock (12) had independently reported striking differences in the carcinogenic activity of 1,2,5,6-dibenzanthracene injected intramuscularly into fowls: With lard half the animals developed tumors; with "egg-yolk fat" or "chicken fat" no tumors appeared. Recently, Murphy and Sturm (9) have found in similar experiments that whereas 96 per cent of tumors were obtained by benzene solutions, the local carcinogenesis with lard (30 per cent of tumors) and chicken fat (10 per cent) was much lower; on the other hand distant tumors and other indications of dispersal (liver damage and leukosis) were observed only with the 2 latter solvents.

Results similar to those of Peacock (12) were reported for mice by Peacock and Beck (13), who used in this series of experiments 0.5 to 1 mgm. of 3,4-benzpyrene given subcutaneously. Whereas the powdered solid substance, or its solution in ethyl ether or in "mouse fat" produced only a few tumors, carcinogenic activity was pronounced when the solvent was olive oil, "mouse lipoids containing fats and sterols," or a mixture of olive oil and paraffin. In this second group, "the solvents used tended to be retained at the site of injection, and also to render the absorption of benzpyrene slower" (13): The solvents of the former group were regarded as producing an effect tantamount to solution in the mouse's own fat.

Morton and Mider (7) obtained only 1 tumor in 44 mice which had received subcutaneously 0.25 mgm. of 3,4-benzpyrene dissolved in a light petroleum extract of mouse carcases, while the same amount of hydrocarbon dissolved in sesame oil produced 36 tumors in 46 mice.[1]

The striking results of Peacock and Beck and Morton and Mider could not be confirmed by Oberling, Guérin, and Sannié (11), Oberling, Guérin, and Guérin (10), or Shimkin and Andervont (17). These authors found no effect of homologous fat, from the rat and mouse respectively, on tumor production by carcinogenic hydrocarbons. Shimkin and Andervont also obtained no special effect when liver fat, brain fat, abdominal fat, chest fat, or fat from the rest of the body of the mouse was used as solvent.

In view of these inconsistencies, we decided to investigate the relation of solvent to carcinogenesis in some detail. The preliminary results were so decidedly in favor of an anticarcinogenic effect of purified mouse fat that although the main program is not yet completed it was thought advisable to present them here. In addition, a similar anticarcinogenic action of ox brain lipoids was found in experiments on mice, showing that extracts from a different species of animal may be as active as homologous fats.

EXPERIMENTAL

Preparation of Lipins and Fats

The methods are reported in detail, since much confusion has arisen from incomplete descriptions (17).

[1] The same authors (8) found an *increased* number of skin tumors when a similar mouse extract was applied to the skin of mice just before painting with a benzene solution of benzpyrene; a similar effect in rats for mixtures of pinene tar and rat tissue extract had previously been described by Watson (20). Baumann and his coworkers (3) found that cholesterol, when dissolved in cottonseed oil but not in benzene, definitely stimulated the rate of tumor production when benzpyrene was painted on the ears of mice. On the other hand, painting a benzpyrene solution in extract of mouse carcases was found by Morton and Mider (8) to give fewer skin tumors than a benzene solution.

Mouse extracts.—Twenty mice were killed, and their tails and intestines removed. The mince was extracted with 1 liter of acetone and left standing overnight at 0°. The extraction was repeated once. Both acetone extracts were combined and retained, as described below. The residual material was dried in a vacuum desiccator and powdered. It was extracted for 6 hours with chloroform-methanol (1:3) in a Soxhlet apparatus. The extract, on standing at 0°, deposited some insoluble material which was removed by filtration. The solvent was removed under reduced pressure in carbon dioxide, and the residue dissolved in 400 ml. light petroleum (boiling point 40–60° C.). After standing overnight at 0°, the extract was filtered, concentrated to 150 ml., and poured into 1 liter of acetone. After having been kept overnight at 0° the precipitate, "mouse lipins," was collected by filtration and dried *in vacuo*. It was free from cholesterol.

The filtrate from the acetone precipitation was combined with the original acetone extracts and shaken with solid calcium chloride. The acetone layer was separated from the aqueous $CaCl_2$ and evaporated. The residue was taken up in ethyl ether, dried over Na_2SO_4, filtered, and evaporated. The residue, constituting the "neutral fat fraction," contained 2 per cent cholesterol. On standing at room temperature it partially solidified, and was separated into "solid mouse fat" and "liquid mouse fat" by filtration. The liquid fraction had 0.07 per cent P and 0.1 per cent N; the phosphorus analysis would indicate a *maximum* content of about 1.5 per cent phospholipins in the fraction.

Ox liver extract.—Two kg. of liver from a freshly killed animal were minced and stirred into 2.5 liters of acetone. After this mixture had stood overnight at 0° and had been filtered, a further extraction with 2 liters of acetone was made, the 2 acetone extracts being rejected. The filtered solid was dried *in vacuo,* powdered, and extracted in a Soxhlet apparatus with chloroform-methanol, as for the mouse extract. Yield 70 gm., "liver lipins"; not further purified.

Ox brain extract.—Two brains from freshly killed oxen were freed from blood and membranes and minced. One kg. of the mince was stirred with 1.3 liters of acetone, left overnight at 0°, filtered, and the extraction repeated. After it had been freed from acetone in a vacuum desiccator, the residue of 200 gm. of dry powder was extracted in a Soxhlet apparatus with chloroform-methanol as for mouse extract. The part insoluble at 0° was filtered off and the solvent removed under CO_2 *in vacuo;* the residue weighed 64 gm. It was combined with the residue from evaporation of the second acetone extract (4 gm.); the combined extracts were taken up in ether (300 ml.); the filtered solution was kept at 0° overnight and again filtered. The ether solution was freed from ether *in vacuo* and the residue, which was a stiff semisolid mass, was dissolved in about 50 ml. hot methanol and chilled to 0°. The filtrate was concentrated to about 20 ml., again chilled, and filtered. The filtrate on evaporation yielded 15 gm. yellowish semisolid residue, ox brain extract.

All the preparations were kept in stoppered bottles at 0° until required for use.

Addition of benzpyrene.—Benzpyrene (1.0 mgm. per cc.) was dissolved directly in the liquid preparations used. A weighed portion of each of the semisolid extracts was thoroughly mixed with an equal volume of 0.1 per cent of 3,4-benzpyrene solution (Messrs. L. Light and Company) in ethyl ether. After evaporation *in vacuo* to remove the ether completely, the residue contained 0.1 per cent of benzpyrene. In the case of the liver lipoids, this treatment caused some hardening of the original material; hence a second batch was prepared in which the lipoids were dissolved in the chloroform-methanol mixture (1:3) containing benzpyrene as above. The residue on removal of the solvent was then not so hard. These 2 preparations are termed liver extracts 1 and 2. Preparation 2 was sufficiently fluid after warming to 50° for use in a syringe as described below. All other animal extracts except liquid and solid mouse fat were implanted as pellets.

Preparation of pellets for implanting.—Pellets were prepared in the following way. A glass tube, provided with a closely fitting glass rod as a plunger, was calibrated by weighing to deliver a cylindrical pellet about 6 mm. in diameter and of 300 mgm. weight, containing 0.3 mgm. of benzpyrene. The average error was about 5 per cent, as determined by numerous control weighings.

Implantation and injection into mice.—Male mice of mixed stock and about 3 months old were used for these experiments. They were anesthetized by intraperitoneal injection of 0.5 ml. avertin, diluted 1:60. If this proved insufficient, a slight inhalation of ether was enough to produce full anesthesia. The fur of the back was clipped, and an incision about 1 cm. long was made in the interscapular skin. With a blunt instrument a subcutaneous pocket was formed, the pellet inserted, and the incision sutured by close interrupted sutures.

In the first experiment with liver lipins 1, continuous sutures were used, but these proved inadequate and considerable leakage of the implanted material occurred. After 6 days sloughs formed which healed later, and this experiment was therefore unsatisfactory.

Leakage was carefully controlled, as described below, in all cases by repeated examination of the wounds,

and in many instances by inspection of the area by filtered ultraviolet light.

Liver lipins 2 were injected through a slit a few millimeters long, into which the tip of the syringe (without needle) containing the warmed extract was inserted. The small incision was then firmly sewn with 3 or 4 separate sutures and painted with collodion. Nevertheless much ulceration was observed in mice injected with this fraction. The question of ulceration in these experiments is discussed below.

Liquid and solid mouse fats were injected in the usual way by means of a syringe and needle, the solid mouse fat being previously warmed, when it readily liquefied completely. The same method was of course used for arachis and sesame oils. With these 4 solvents the benzpyrene was directly dissolved in the warmed oils.

All injections and implants were made subcutaneously in the interscapular region. In all experiments each animal received a single dose of 0.3 mgm. of 3,4-benzpyrene dissolved in 0.3 cc. of the solvent, or in 300 mgm. of the semisolid fractions.

Leakage, Ulceration, and the Presence of Subcutaneous Lumps

In the following account, leakage or suspected leakage of injected material has been described as primary, secondary, or nil.

Primary leakage indicates the incomplete healing of the incision, or inadequate suturing of the wound such that the injected material was suspected to have escaped wholly or in part. Frequently, but not always, this could be confirmed by examination in the ultraviolet.

Secondary leakage means that the incision was adequately sutured, the wound had healed, but that later ulceration occurred. With several of the solvents subcutaneous lumps were formed at or near the injection site, as discussed below, and these frequently persisted for long periods. These lumps were either single or multiple, developed a few days after injection, and were round and resilient. If ulceration occurred, this was usually at the apex of the lump. Such secondary ulceration usually occurred a week to a fortnight after insertion. Following such ulceration the lump often disappeared, presumably by leakage through the ulcer.

Results

The first tumor appeared on the 76th day, and only 5 mice died before this time; these are omitted from consideration in assessing tumor incidences. The experiment was concluded after 40 weeks. The numbers of mice used, the times of survival of nontumor mice, and leakage or suspected leakage are shown in Table I. Times of survival are not shown for tumor animals, as they were killed for autopsy as soon as the growth of the tumor was clear.

Table I: Numbers of Mice Injected with 3,4-Benzpyrene, Leakage of Injected Material, and Survival

Solvent	Number of mice	Number alive at time of 1st tumor (day 76)	Number of tumor mice	Leakage in mice surviving more than 76 days			Time of death of non-tumor mice, weeks	Number surviving * at 40th week
				Primary	Secondary	Nil		
Sesame oil	20	20	18	0	0	20	11	1
Arachis oil	20	20	12	0	0	20	13, 13, 15, 18, 29, 32	2
Liquid mouse fat	28	28	4	0	7	21	14	23
Solid mouse fat	16	14	2	0	7	7	12, 21, 23, 35	9
Ox brain	30	29	1	2	3	24	11, 12, 23, 27, 29, 30, 36	21
Mouse lipins	11	10	0	0	9	1	15, 18, 21, 21, 23, 27, 38	3
Liver (1)	23	22	1	13	1	8 †	12, 20, 33, 39	17
Liver (2)	30	30	0	0	28	2	13, 13, 14, 14, 25, 25, 34, 35	22

* Tumor-bearing mice killed for autopsy.

† Leakage in this 1st series may have escaped detection in some of these 8 mice.

The numbers and times of appearance of tumors with each extract are shown in Table II, and the results with individual solvents are described hereafter.

Sesame oil.—Eighteen tumors arose in 20 mice. No subcutaneous lumps were present after injection, and none developed until the tumors appeared. There was no evidence of leakage of the injected material. The latent time had a range of 11 to 38 weeks with a distribution such that the median was at 17 weeks, with a decided tendency for the later occasional occurrence of isolated tumors spread over the period from the 19th to the 38th week (see Table II).

Arachis oil.—Twelve tumors arose in 20 mice, again without formation of any preceding subcutane-

ous lumps and with no evidence of any leakage. The latent time had a range of 11 to 25 weeks, distributed with the median at 19 weeks, and fewer later tumors appeared with this series. Table II seems to indicate a slight tendency towards later appearance of the main yield of tumors, in comparison with sesame oil. The tumors were also less numerous.

If this difference in tumor incidence with these 2 solvents is a real one, it should be considered in relation to the response of the mice to the injection of the oils alone. According to Shimkin and Grady (18), sesame oil is absorbed almost completely within a month, while "peanut oil was tolerated poorly, in that it was not absorbed, and produced huge oil deposits, local tissue reaction, and abscesses, which did not recede for as long as 11 months after the last injection."

We ourselves did not observe any abscess or any pronounced tissue reaction with the sample of arachis oil (or of sesame oil) and with the mice used. In animals which died or were killed

TABLE II: TUMOR INCIDENCE

Solvent	Number of mice alive on 76th day	Latent period of the tumors, weeks
Sesame oil	20	11, 15, 15, 15, 15, 15, 17, 17, 17, 19, 21, 21, 25, 28, 29, 31, 37, 38
Arachis oil	20	11, 11, 17, 18, 19, 19, 19, 19, 23, 23, 25, 25
Liquid mouse fat	28	16, 16, 23, 29
Solid mouse fat	14	16, 16
Ox brain	29	11
WITH SERIOUS LEAKAGE		
Mouse lipins	10	None
Liver (1)	22	16
Liver (2)	30	None

(from the 11th week onward) no oil deposits were found at autopsy.

Certainly there was no great difference between the carcinogenic effects of benzpyrene solutions in these 2 oils, such as might have been expected on the basis of Shimkin and Grady's experience, if the rate of absorption of the solvent were indeed a determining factor as Peacock and others have suggested. On the contrary, the tendency to a *lesser* tumor incidence with the supposedly more persistent arachis oil seems to be opposed to this view.

Ox brain lipins.—Only one tumor arose in 30 mice injected with this solvent, and this appeared after 76 days, being in fact the earliest of the whole series. It was situated behind the left ear, and microscopically was a typical spindle cell sarcoma. Three mice, which have been omitted from the tables, died shortly after injection, on the 2nd and 3rd days, and in these the inoculated material had liquefied and was spread in a thin layer over the greater part of the subcutaneous tissues of the back. This layer, the gall bladder, and the bile, all showed strong fluorescence. There was no indication of infection, and the subcutaneous tissues looked quite normal. Lumps near the injection site were present in only 7 mice and persisted from 3 to 6 weeks. There appeared to be no connection between the presence of these lumps and the formation of tumors, since the one mouse which later developed a sarcoma showed no sign of lump formation following the injection. In one mouse which died after 7 weeks no trace of the injected pellet and no fluorescent material were present. Occasional reddening of the skin and later baldness were observed. The incisions generally healed rapidly, and primary leakage occurred in only 2 mice; in the remaining 27 mice, 24 perfectly healed wounds showed no sign of ulceration or leakage of injected material. In the other 3 mice secondary leakage was suspected. In spite of the fact that 20 mice in which there was no primary leakage survived for more than 33 weeks, no neoplasm appeared other than the sarcoma mentioned above.

Liquid mouse fat.—Tumors arose in 4 of the 28 mice of this series during the 16th to 29th weeks. Subcutaneous lumps, which were occasionally double or treble, appeared after injection in 24 of the mice, and persisted for periods of from 4 to 22 weeks. Baldness near these lumps was fairly frequent. The bald areas in this series were fluorescent in the ultraviolet beam, even when the skin appeared to be quite intact. The lump persisted in one mouse up to the time of appearance of the growing tumor; it was first detected as a small hard pellet in the middle of the left side 1 week after the injection. It gradually increased in size for 8 weeks, and thereafter diminished until after 13 weeks it was again small. At this stage it began to grow rapidly, and at autopsy a tumor 1.5 cm. in diameter was present. In the 3 other tumor mice, the subcutaneous lumps persisted up to 5, 6, and 12 weeks before the tumor became palpable; in the interim the original lumps had completely regressed, to be replaced later by growing tumors.

No instance of primary leakage occurred with this material, but in 7 mice there was evidence of secondary leakage. Twenty-one mice survived more than 11 weeks with no detectable leakage. The absence of leakage in these was in every case supported by the persistence of the subcutaneous lumps.

The tumors were local sarcomas, except one intrathoracic tumor, 1.5×1.2 cm., which was a lymphoblastoma infiltrating the walls of vessels and the submucosa of a bronchus.

Solid mouse fat.—Only 2 of the 16 mice injected had tumors, although there was no primary leakage, and all the animals had subcutaneous lumps near the site of injection which persisted for from 3 to 17 weeks. In the 2 tumor mice these lumps persisted right up to the time when a tumor developed and began to grow rapidly (15th week). However, at least one other mouse bore a lump up to the 17th week, without subsequently developing any tumor.

Two mice died after 4 weeks, and in these no trace of the injected material could be detected at autopsy. Secondary leakage may have occurred in 7 of the mice, since in these animals small ulcers were observed after the primary healing, but the presence of lumps in all the mice may perhaps be regarded as an indication of no appreciable leakage and as showing sufficient persistence of the local depot. The elimination of the injected material does not therefore appear to be the cause of the low incidence of tumors with either liquid or solid mouse fat.

In one mouse, which 3 days after injection had a large subcutaneous lump, 2 separate lumps developed from this on the back near the scar 10 days later. The smaller of the 2 regressed, while the larger remained unchanged for 14 weeks, when it was replaced by a growing tumor. Eight weeks later this had become 2 × 1 cm. and the animal was killed. At autopsy, a thick walled cyst containing blood, fibrin, and necrotic tumor cells was found, the base of which consisted of a spindle cell sarcoma. The appearances suggested encapsulation of the injected material and its subsequent replacement by hemorrhagic fluid, accompanied by development of the neoplasm in the cyst wall.

Mouse lipins.—No tumor was observed in any of the 11 mice injected, but with this material leakage was suspected in 9 of the animals. Primary leakage did not occur, and all the mice had large fluctuant lumps, which persisted only about 1 week after implantation. The secondary ulceration, however, was sufficiently severe to render the experiment with this material inconclusive. In one mouse which died 6 days after injection part of the pellet was still present in its original state, but most was liquefied and spread out over the scapula. There were indications of encapsulation and local tissue reaction. No fluorescence was observed in the bile of this mouse.

Liver extract 1.—As already explained, the continuous sutures used in this, the first experiment of the series to be made, were a point of weakness. Definite or suspected primary leakage occurred in 13 of 23 mice, and secondary leakage in one animal. One mouse had a lump which persisted for 12 weeks. The contamination of the skin around the ulcers probably accounted for the transient presence of a number of small warts which were seen in several animals. With one exception these regressed again after a few weeks. The exception appeared as a papilloma 9 weeks after injection, and after a further 15 weeks it began to increase in size and resembled an epithelioma. Histological examination showed that it consisted of a squamous papilloma, one side of which had developed into a superficial but active, well differentiated, moderately keratinizing, squamous cell carcinoma. This observation confirms the probability of leakage of injected material, with consequent skin contamination. One animal, dying 9 weeks after injection, had a swelling in the mid-back which was microscopically shown to be inflammatory and not neoplastic.

Liver extract 2.—In this series the same care was taken as in all the other experiments, except that with liver extract 1, to ensure that the incision was thoroughly sutured. This resulted in the elimination of all primary leakage in this series. Nevertheless, secondary ulceration occurred in no less than 28 of the 30 mice used. In 23 of these leakage was definitely proved, and could easily be confirmed by inspection in the ultraviolet; in 5 others secondary leakage was suspected but could not be definitely proved. Hence only 2 of this series survived for a sufficiently long period without proved or suspected leakage. Sixteen mice had subcutaneous lumps, which persisted for only 1 to 2 weeks, and baldness was frequent. No lesion attributable to the carcinogen was observed in any mouse.

DISCUSSION

The ensuing discussion will be confined to those solvents which caused no ulceration, or so little that it seems improbable that leakage of benzpyrene and solvent could be held responsible for the results observed. This excludes from consideration mouse lipins, and both the liver extracts employed.

It may be noted in passing that Morelli and Dansi (6) observed that with the mixture of rat lipins and rat fats used in their experiments "a formation of ulcers earlier than in the case of lard was also observed (10 to 15 days)." Other workers do not mention the occurrence of ulceration in similar experiments.

The results described confirm and extend the observations of Peacock and Beck (13) and Morton and Mider (7) that the use of homologous fat, *i.e.* that extracted from the same species, as a solvent for benzpyrene greatly reduces the incidence of tumors resulting from subcutaneous injection of the solution into mice. In our experiments purification by removal of phospholipins and separation into solid and liquid fractions did not remove this property of the fat.

These results appear to be contradictory to those of Oberling and his associates (11, 10) and of Shimkin and Andervont (17). In seeking an explanation of the discrepancy, an important and perhaps decisive factor may be the quantity and activity of the carcinogen injected. Sall and Shear (14) have rightly urged the desirability of using sufficiently small doses in all experiments designed to demonstrate a cocarcinogenic or anticarcinogenic activity. These authors found that for this purpose about 0.1 mgm. of 3,4-benzpyrene or 1,2,5,6-dibenzanthracene was a suitable amount for subcutaneous injection into mice, but with methylcholanthrene the useful dose had to be reduced to 0.02 mgm., otherwise any effect of the added substance might be masked by the excessive activity of the carcinogen.

Many of the results of Oberling and his coworkers were obtained with large doses (75 to 25 mgm.) of benzpyrene injected into rats, and for the reason given above it is not certain that any effect of rat fat as a solvent was to be expected; in fact none was found. In the one experiment in which they used smaller quantities (1 mgm.), these authors (11) also observed

no effect of the solvent (5 tumors in 8 surviving rats injected with benzpyrene in rat fat; 11 tumors in 14 rats which received the same dose in olive oil). Unfortunately, in their second paper (10) the results for 1 mgm. doses in olive oil and in rat fat are not given separately, but are grouped together. Since, however, the authors do not comment on the result observed, it is probable that this confirmed their earlier finding that, in the rat, no difference in tumor incidence resulted from the use of homologous fat as a solvent instead of oil or lard.

If Shimkin and Andervont's observations are considered from the same standpoint, it seems likely that the quantity of carcinogen used in nearly all their experiments (0.5 to 1.0 mgm. of methylcholanthrene) may have been great enough to mask any inhibitory effect of the solvent, especially as the C3H mice used are known to be highly susceptible to the induction of tumors by subcutaneous injection. This is supported by the large proportion of tumors which arose even in the mice receiving the smallest dose (0.5 mgm. of methylcholanthrene), amounting to 168 tumors among 187 animals. Shimkin and Andervont themselves call attention to the fact that it is remarkable that even with these relatively large doses of methylcholanthrene the effect of variation in the composition of the lard, or of the particular pure compound used as solvent, should have been reflected in different tumor incidences. In Shimkin and Andervont's experiments, differences in the numbers of tumors produced were less notable than differences in the latent times. In contrast with these results, we found great differences in the *numbers* of tumors produced before the conclusion of the experiment, in its 9th month. In the few tumors which we obtained by the use of homologous solvents, the latent period was normal. In the single tumor produced with brain extract as solvent, the latent period was even unusually short.

On the basis of Sall and Shear's assessment, the 0.3 mgm. dose of benzpyrene used in the present experiments would be of an activity comparable with that of 0.06 mgm. of methylcholanthrene. With this dosage of benzpyrene we observed a definite inhibitory effect of liquid mouse fat on carcinogenesis. The most probable assumption is that had we used doses comparable with those of Shimkin and Andervont (*viz.*, 2.5 to 5.0 mgm. in the case of benzpyrene) we too might have failed to observe the inhibitory effect. This point is being tested.

Peacock and Beck (13) attribute the anticarcinogenic action of homologous fat to the more rapid elimination of the solvent from the injection site, and the consequently more rapid absorption of the benzpyrene, as indicated by visual inspection of the fluorescence in this region. Without necessarily contradicting this explanation, our experiments are not easily reconcilable with it in 2 respects: (a) the persistence near the injection site of the liquid mouse fat of subcutaneous lumps, apparently indicating the very slow dispersion of the injected solution; (b) the fact that the solid fraction from mouse fat, which might be expected to be even less readily dispersed, and which also gave rise to many persistent lumps, was similarly effective in preventing tumor formation. Morelli and Dansi (6) have encountered a similar difficulty in ascribing to the more rapid elimination of the solvent the suppression of the inhibitory power of the carcinogen on tumor growth observed by them when benzpyrene or dibenzanthracene was injected into tumor-bearing rats in the form of a solution in "mixtures of homologous lipoids and fats." In fact, they report that in those animals that died because of tumor growth "most of the fat remained at the site of injection." This question we hope to settle by quantitative experiments, now in hand, on the elimination of the carcinogen.

The results with ox brain lipins reported in this paper show that an inhibitory effect on carcinogenesis, similar to that of homologous solvents, may also be obtained by the use of lipins from another species of animal. The brain extract used, like the sesame and arachis oils employed for comparison, was quite fluid and mobile within the animal, spreading over a large area of the subcutaneous tissue of the back. Nevertheless, while the use of the 2 vegetable solvents resulted in numerous tumors, the carcinogenic activity of benzpyrene was almost completely suppressed when the solvent was brain lipoids. Further investigation of this effect, by the employment as solvents of various purified lipins, especially cephalin and lecithin, and by measurements of elimination and dispersal of the carcinogen, are now in progress.

SUMMARY

1. The subcutaneous injection into mice of solutions of benzpyrene in sesame oil or in arachis oil gave rise to tumors in a large proportion of the animals. The same dose of benzpyrene dissolved in either the liquid or the solid fraction of mouse fat, which had been purified and freed from phospholipins, produced only a few tumors. This result confirms and extends those of Peacock and Beck (13) and Morton and Mider (7). Possible reasons for different results obtained by others are discussed.

2. It is not necessary for the suppression of carcinogenic activity that the lipoid material should be of homologous origin. A mixture of fats and lipins obtained from ox brain was equally effective.

3. Conclusive results could not be obtained with the ox liver extract or with the mouse lipins employed, owing to the prevalence of ulceration and consequent leakage of the injected material. Examination of the mouse in the ultraviolet beam is usually a valuable aid towards detection of such leakage, but when mouse fat was the solvent the apparently intact skin also fluoresced.

4. In such experiments the amount of carcinogen should not be too great, or any inhibitory tendency may be masked by its excessive activity.

We wish to thank Professor A. F. Bernard Shaw and Dr. J. G. Thomson, Pathology Department, University of Durham, for histological examination of material.—Authors.

REFERENCES

1. Andervont, H. B., and Lorenz, E. Dibenzanthracene Tumors in Mice. The Production of Subcutaneous, Pulmonary, and Liver Tumors by Serum Dispersions and Lard Solutions. Pub. Health Rep., **52**:637-647. 1937.
2. Athias, M., and Furtado-Dias, M. T. Sarcome transplantable du rein provoqué par le méthylcholanthrène chez le rat. Compt. rend. Soc. de biol., **127**:237-238. 1938.
3. Baumann, C. A., Rusch, H. P., Kline, B. E., and Jacobi, H. P. Does Cholesterol Stimulate Tumor Development? Am. J. Cancer, **38**:76-80. 1940.

4. Chalmers, J. G., and Peacock, P. R. Further Evidence Regarding the Elimination of Certain Polycyclic Hydrocarbons from the Animal Body. Biochem. J., **30**:1242-1248. 1936.
5. Dunning, W. F., Curtis, M. R., and Bullock, F. D. The Respective Rôles of Heredity and Somatic Mutation in the Origin of Malignancy. Am. J. Cancer, **28**:681-712. 1936.
6. Morelli, E., and Dansi, A. Variations in the Inhibitory Power of Carcinogenic Hydrocarbons According to the Solvent. Nature, London, **143**:1021. 1939.
7. Morton, J. J., and Mider, G. B. Effect of Petroleum Ether Extract of Mouse Carcasses as Solvent in Production of Sarcoma. Proc. Soc. Exper. Biol. & Med., **41**:357-360. 1939.
8. Morton, J. J., and Mider, G. B. Effect of Petroleum Ether Extract of Mouse Carcasses on Skin Tumor Production in C57 Black Mice. Pub. Health Rep., **55**:670-676. 1940.
9. Murphy, Jas. B., and Sturm, E. Further Investigation of Induced Tumors in Fowls. Cancer Research, **1**:477-483. 1941.
10. Oberling, C., Guérin, M., and Guérin, P. Particularités évolutives des tumeurs produites avec de fortes doses de benzopyrène. Bull. Assoc. franç. p. l'étude du cancer, **28**:198-213. 1939.
11. Oberling, C., Guérin, M., and Sannié, C. Influence du solvant sur le pouvoir cancérigène du 3-4 benzopyrène. Compt. rend Soc. de biol., **130**:17-19. 1939.
12. Peacock, P. R. Proc. Leeuwenhoek Vereeniging, Amsterdam, June, 1935. Cited by Chalmers and Peacock (4).
13. Peacock, P. R., and Beck, S. Rate of Absorption of Carcinogens and Local Tissue Reaction as Factors Influencing Carcinogenesis. Brit. J. Exper. Path., **19**:315-319. 1938.
14. Sall, R. D., and Shear, M. J. Studies in Carcinogenesis. XII. Effect of the Basic Fraction of Creosote Oil on the Production of Tumors in Mice by Chemical Carcinogens. J. Nat. Cancer Inst., **1**:45-55. 1940.
15. Shear, M. J., and Lorenz, E. Studies in Carcinogenesis. VI. Hydrocarbon-Cholesterol Pellets in Albino Mice. Am. J. Cancer, **36**:201-210. 1939.
16. Shear, M. J., and Ilfeld, F. W. Studies in Carcinogenesis. IX. Hydrocarbon-Cholesterol Pellets in Strain D Mice. Am. J. Path., **16**:287-293. 1940.
17. Shimkin, M. B., and Andervont, H. B. Factors Influencing Carcinogenesis with Methylcholanthrene. III. The Effect of Solvents. Pub. Health Rep., **55**:537-545. 1940.
18. Shimkin, M. B., and Grady, H. G. Carcinogenic Potency of Stilbestrol and Estrone in Strain C3H Mice. J. Nat. Cancer Inst., **1**:119-128. 1940.
19. Strong, L. C., and Smith, G. M. Local Induction of Carcinoma of Mammary Gland by Methylcholanthrene. Yale J. Biol. & Med., **11**:589-592. 1939.
20. Watson, A. F. Comparative Studies on Carcinogenesis in Rats. Am. J. Cancer, **25**:753-762. 1935.

Studies in Esterase (Butyric) Activity

I. Esterase Content of Serum of Mice from Certain Cancer-Resistant and Cancer-Susceptible Strains*

V. R. Khanolkar, M.D., and R. G. Chitre, Ph.D.

(From the Tata Memorial Hospital for the Treatment of Cancer and Allied Diseases, Bombay, India)

(Received for publication April 21, 1942)

It was discovered by Cherry and Crandall (2) in 1932 that an enzyme was present in the blood of dogs which was different from lipase. It was capable of splitting the esters of lower fatty acids with monohydric alcohols. This enzyme had no action on esters of the trihydric alcohol, glycerol. The enzyme was called esterase to distinguish it from lipase. Since its discovery investigations have been carried on concerning the presence of this enzyme in health and disease. Mosters (9) and Gajdos (6) studied the influence of vitamin C on its blood level. Cajori and Vars (1) studied the effect of chloroform anesthesia, and Forbes and his associates (4, 5) observed the effects of injection of certain chemicals and of high fat and low choline diets.

There have been very few investigations on the content of esterase in the serum in malignant disease and the significance of the findings is somewhat obscure. Green (7) found that in rats during the growth of Jensen sarcoma the esterase activity of the serum showed a pronounced reduction and that a parallel fall occurred in some of the tissues. The reduction in the serum esterase content started "at an early stage of the tumor growth, when the animal appeared vigorous and healthy." In animals resistant to inoculation of the sarcoma the serum esterase showed a tendency to rise. In human disease, however, the results were different and in cancer patients with a good general health the serum esterase was on an average slightly greater than in a noncancerous normal person. Troescher and Norris (11) have shown that in rats bearing transplanted adenocarcinoma of the breast esterase activity in the serum decreased and tended to rise towards the normal level when the tumor regressed as a result of x-ray treatment. These findings suggest that the changes in esterase activity of the serum are an expression of some metabolic disturbance in the body which accompanies the growth of the malignant tumor, and that it forms a part of the abnormal physiology associated with the presence of cancer or precancerous lesions. This is further borne out by the fact that strains of animals which are normally resistant to tumor growth show a significant fall in serum esterase after a successful transplantation. It was therefore decided to find out if any difference existed between the serum esterase content of healthy animals from strains which have a low spontaneous cancer incidence and from those with a very high incidence. It is believed that such an investigation would establish standards for further work on the level of this enzyme in blood and other tissues during the development of a malignant growth.

EXPERIMENTAL

Material.—For the purpose of this investigation 3 highly inbred strains of laboratory bred mice were used: (a) the C57 black, which is resistant to cancer; (b) C3H, which develops carcinoma of the mammary glands; and (c) strain A, in which the females develop carcinoma of the breast and the males develop carcinoma of the lungs.[1] Under laboratory conditions in the United States of America the incidence of spontaneous mammary gland cancer in breeding mice of C57 strain is virtually nil and in C3H and A strains 95 to 100 per cent and 80 to 85 per cent respectively (10). All the strains received the same complete stock diet, similar to that recommended by Dr. Slanetz at the Department of Animal Care in the Laboratories of Columbia University, College of Physicians and

* Strain C57 black was obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, through the kindness of Dr. C. C. Little, and C3H and A strains were obtained from the Department of Anatomy, Yale University School of Medicine, New Haven, Conn., through the kindness of Dr. Leonell C. Strong.

[1] As proof could not be sent to the authors because of the war, the Editorial Committee takes the liberty of adding the following note:

In American laboratories equal numbers of males and females belonging to strain A develop spontaneous pulmonary tumors, and these growths are not carcinomas but adenomas (*cf.* Shimkin, M. B. Induced Pulmonary Tumors in Mice. II. Reaction of Lungs of Strain A Mice to Carcinogenic Hydrocarbons. Arch. Path., **29**:239-255. 1940).

Surgeons, New York, N. Y. From each strain 36 mice were used. They belonged to 2 different age groups. The first group, between 6 and 8 months, included 24 animals of each strain and the second, between 8 and 10 months, was composed of 12 animals from each. was added as an indicator. If the hydrogen ion concentration of the mixture was disturbed in this process it was readjusted to 8.9, and the mixture was incubated at 37.5° C. for 6 hours. A blank control tube was included in every case, containing all the solutions

TABLE I

A strain			C57 strain			C3H strain		
Number	Sex	N/100 NaOH, cc.	Number	Sex	N/100 NaOH, cc.	Number	Sex	N/100 NaOH, cc.
			AGE GROUP 6 TO 8 MONTHS					
74	F	40.5	26	F	19.0	44	F	43.0
75	F	32.0	27	F	15.5	45	F	43.0
76	F	30.5	28	F	15.0	46	F	46.0
77	F	35.5	29	F	20.0	47	F	50.0
78	F	28.5	30	F	16.0	48	F	43.0
79	F	30.5	31	F	17.0	49	F	33.0
98	F	47.5	50	F	13.0	6	F	35.5
99	F	34.5	51	F	16.0	7	F	32.0
100	F	33.5	52	F	15.5	8	F	28.0
101	F	36.0	53	F	12.5	9	F	34.5
24	F	32.0	54	F	15.0	10	F	31.0
25	F	36.5	55	F	12.5	11	F	32.5
80	M	36.0	32	M	14.0	12	F	43.0
81	M	36.0	33	M	14.5	38	M	39.0
82	M	33.0	34	M	13.0	39	M	27.5
83	M	38.5	35	M	19.0	40	M	30.0
84	M	33.0	36	M	21.0	41	M	27.0
85	M	30.0	37	M	19.0	42	M	37.0
102	M	37.0	56	M	16.5	43	M	34.5
103	M	37.5	57	M	18.5	106	M	33.5
104	M	35.0	58	M	17.0	107	M	34.5
105	M	36.0	59	M	17.0	108	M	35.5
20	M	44.0	60	M	14.0	109	M	35.0
21	M	36.5	61	M	22.0	110	M	36.5
			AGE GROUP 8 TO 10 MONTHS					
92	F	36.0	129	F	17.0	117	F	32.0
93	F	32.0	130	F	17.5	118	F	33.5
94	F	41.0	131	F	17.5	119	F	34.0
95	F	32.0	132	F	18.0	120	F	32.0
96	F	35.0	133	F	19.5	121	F	38.5
97	F	45.5	134	F	14.0	122	F	36.0
86	M	45.5	123	M	16.5	111	M	38.0
87	M	36.5	124	M	15.0	112	M	32.0
88	M	31.0	125	M	18.0	113	M	35.0
89	M	40.0	126	M	16.0	114	M	50.0
90	M	36.5	127	M	17.0	115	M	30.0
91	M	40.5	128	M	19.0	116	M	38.5
Mean		36.2	Mean		16.6	Mean		35.9
Maximum		47.5	Maximum		22.0	Maximum		50.0
Minimum		28.5	Minimum		12.5	Minimum		27.0
Standard error		±0.59	Standard error		±0.39	Standard error		±0.94

There were almost an equal number of males and females in both groups.

Determination of serum esterase.—Animals were killed by chloroform; about 1 cc. of blood was drawn from the portal vein into a glass syringe and the serum was separated in the usual manner. One-tenth cc. of serum was mixed with 5 cc. of phosphate buffer, pH 8.9, and a few drops of ethyl butyrate. Phenolpthalein with the exception of the serum. The liberated butyric acid was titrated against N/100 NaOH. The amount of N/100 NaOH required for the neutralization of the liberated acid was taken as a measure of esterase activity. Results were expressed in cubic centimeters of N/100 NaOH required to neutralize the acid liberated by 0.5 cc. of serum.

Results.—Table I shows the titer of the esterase

activity in the serum of different strains of mice. The results have been statistically treated according to the method suggested by Hill (8). The data are graphically represented in Fig. 1, which demonstrates the differences between the esterase activity of the blood serum in these 3 strains of mice.

DISCUSSION

The mean values for the esterase content in the serum of susceptible strains A and C3H are 36.2 ± 0.59 and 35.9 ± 0.94 respectively. Both these values are significantly higher than that for the resistant strain, has been recently reported by Figge and Strong (3) shows similar although reversed differences. They have not, however, reported the blood content of this enzyme in the 2 strains of mice studied by them (C3H and JK). These 2 enzyme studies tend to show that the performance of the liver in the cancer-susceptible and cancer-resistant strains of mice investigated is widely different, and that this fact may perhaps be associated with the difference in their susceptibility to cancer. Although a certain amount of evidence is accumulating that the liver plays an important role in the altered metabolism associated with cancerous conditions, the

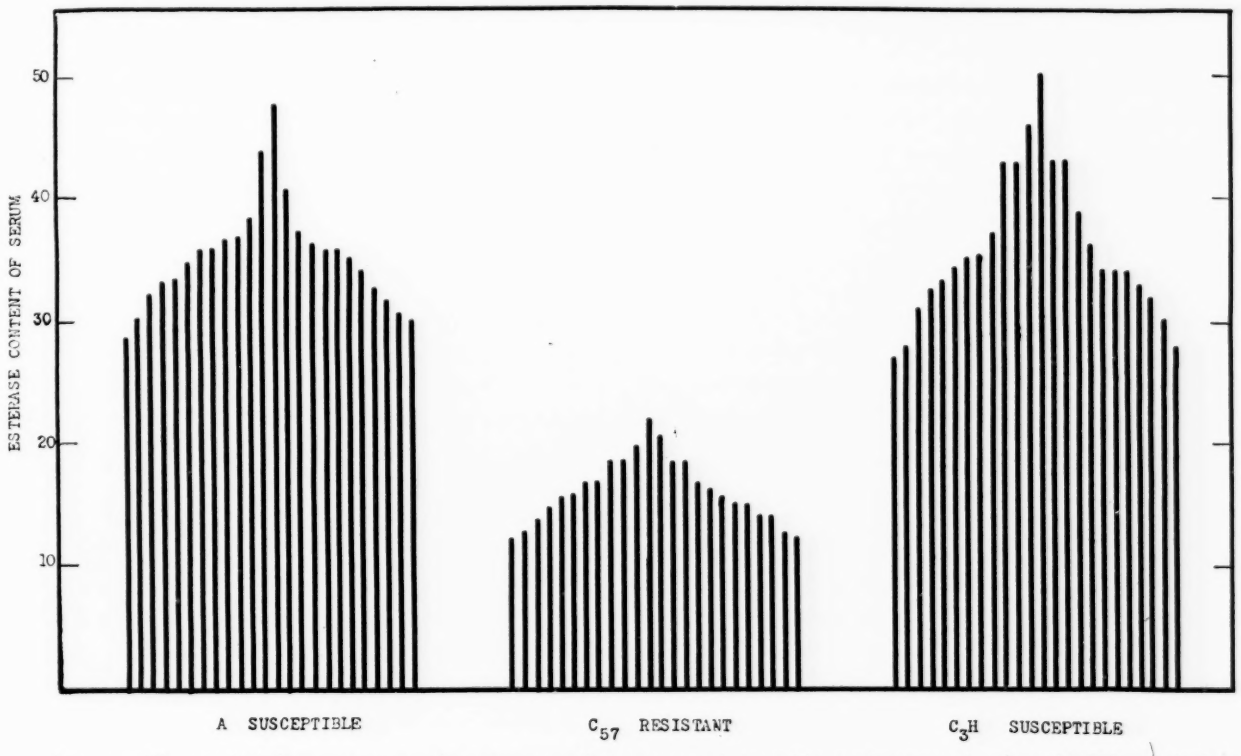

FIG. 1.—The vertical lines represent the esterase activity expressed in cubic centimeters of N/100 NaOH required to neutralize the acid liberated by 0.5 cc. of serum in individual mice of the A and C3H cancer-susceptible and C57 cancer-resistant strains of mice. The lines are arranged to show the normal variation in each group.

C57 black, namely 16.6 ± 0.39. No significant difference was observed in the esterase activity between the 2 age groups and the 2 sexes from the same strains. There are only 2 possible suppositions to explain this difference. Either the resistant strain does not manufacture this enzyme to the same extent in the liver as the susceptible strain, or both strains manufacture the enzyme in equal amount but release it in different quantities into the blood stream. Preliminary studies carried out in this laboratory suggest that the latter supposition is more likely. It has been found in this laboratory that in the experiments conducted so far the livers of C57 mice are more active as regards this enzyme than those of either A or C3H. The enzyme activity of livers in the case of xanthine oxidase which exact nature of the altered metabolism awaits further study and elucidation.

SUMMARY AND CONCLUSIONS

The esterase activity in the serum of 2 strains of cancer-susceptible mice, A and C3H, and one strain of cancer-resistant, C57 black, has been determined.

1. It was found that the activity in the cancer-susceptible strains (36.2 ± 0.59 and 35.9 ± 0.94) was higher than that in the cancer-resistant strain (16.6 ± 0.39). The difference is statistically significant.

2. No difference was noticed in the serum esterase of the 2 age groups, and of the males and females of the same strain.

3. The possible significance of liver metabolism in determining the susceptibility towards cancer is discussed.

REFERENCES

1. Cajori, F. A., and Vars, H. M. The Effect of Chloroform Anesthesia on Serum Amylase and Liver Esterase. Am. J. Physiol., **124**:149-154. 1938.
2. Cherry, I. S., and Crandall, L. A., Jr. The Specificity of Pancreatic Lipase: Its Appearance in the Blood after Pancreatic Injury. Am. J. Physiol., **100**:266-273. 1932.
3. Figge, F. H. J., and Strong, L. C. Xanthine Oxidase (Dehydrogenase) Activity in Livers of Mice of Cancer-Susceptible and Cancer-Resistant Strains. Cancer Research, **1**:779-784. 1941.
4. Forbes, J. C., Outhouse, E. L., and Leach, B. E. Effect of Foods on Serum Esterase of Rats. Proc. Soc. Exper. Biol. & Med., **43**:523-528. 1940.
5. Forbes, J. C., and Outhouse, E. L. Studies on the Mechanism of the Protective Action of Xanthine against Carbon Tetrachloride Poisoning. J. Pharmacol. & Exper. Therap., **68**:185-193. 1940.
6. Gajdos, A. Action de l'acide ascorbique sur le taux lipasique du sérum sanguin. Compt. rend. Soc. de biol., **131**:59-61. 1939.
7. Green, H. N. Changes in Esterase and Fat Content of the Serum Induced by Cancer and Cancer-Producing Agents. Brit. J. Exper. Path., **15**:1-14. 1934.
8. Hill, A. B. Principles of Medical Statistics. Pp. 171. London: The Lancet Limited. 1939.
9. Mosters, J. Über Blutesterasesteigerungen nach peroralen Ascorbinsäuregaben. Klin. Wchnschr., **15**:1557-1560. 1936. Quoted from Forbes *et al.* (4).
10. Suntzeff, V., Kirtz, M. M., Blumenthal, H. T., and Loeb, L. The Incidence of Mammary Gland Carcinoma and Cancer Age in Mice Injected with Estrogen and in Noninjected Mice of Different Strains. Cancer Research, **1**:446-456. 1941.
11. Troescher, E. E., and Norris, E. R. A Micro Blood Esterase Determination Applied to Studies of Rats Bearing Adenocarcinoma. J. Biol. Chem., **132**:553-557. 1940.

Spontaneous Recovery from Sarcoma in Castrated Adult and in Sexually Immature Mice

Ludwik Gross, M.D.

(From The Institute for Medical Research, Christ Hospital, Cincinnati, Ohio)

(Received for publication April 6, 1942)

Recent studies have demonstrated that female mice are considerably more resistant to sarcoma 37 than are male animals. Thus tumors produced by intradermal implantation of this sarcoma underwent spontaneous regression much more frequently in female than in male mice (4, 5). The present studies, a continuation of this previous work, were designed to determine whether the difference in resistance is directly related to either the presence or functioning of the ovaries and testes. This report concerns experiments on castrated and sexually immature animals.

EXPERIMENTAL

The Resistance of Castrated Mice

Bilateral castration of 176 male and 191 female mice[1] was performed under nembutal and ether anesthesia. Sixty of the males and 68 of the females were 21 to 28 days old at the time of castration; the remaining animals were 50 to 60 days old.

Five to 10 weeks after castration,[2] the animals were inoculated intradermally with 0.02 to 0.03 cc. of a 20 per cent suspension of sarcoma 37. The small wheal which resulted from inoculation was covered immediately with a drop of collodion so as to prevent escape of the injected suspension. The technic of preparing and injecting the tumor cell suspension has been described elsewhere (4). The suspensions used in the current work were prepared from 8- to 10-day old subcutaneous tumors from a line which had been transplanted under the skin of young, adult, virgin females every 8 to 10 days for the past 14 months in order to keep the virulence of the growth at a relatively constant level. Each suspension used in this work was tested for bacterial sterility.

Normal adult[3] male and female mice, serving as controls, were inoculated at the same time as the castrated animals, the same suspension and dose being used in both groups. Tumors developed within 10 days following inoculation in all but 9 of the castrated and 14 of the control mice. These 23 animals were discarded and were not included in the tables.

The tumors either grew progressively and killed their hosts or persisted only temporarily,[4] disappearing within 15 to 35 days following inoculation. As Table I shows, the incidence of spontaneous regression was almost identical in the total series of castrated males and females (27 and 24 per cent, respectively). This finding was in contrast to that obtained with the normal control animals, in which tumors regressed in 42 per cent of the females and in only 14 per cent of the males. In comparing the experiments with castrated and normal animals, it should be noted that in most groups the percentage of regressing tumors was higher in castrated than in normal males. On the other hand, the incidence of regressing tumors was lower in castrated than in normal females.

The Resistance of Immature Suckling Mice

In this experiment, groups of immature, suckling mice, 0 to 18 days old, were inoculated intradermally with sarcoma, using the same technic of inoculation and the same dose of tumor suspension as described above. Groups of adult male and female mice were inoculated simultaneously to serve as controls.

Cutaneous tumors appeared within 10 days following inoculation in all immature and control animals. In some of the immature mice, the tumors grew progressively, killing these animals in from 13 to 55 days. The adult animals in which the tumors grew progressively lived somewhat longer than the suckling mice, the average survival time being 39 and 47 days for adult males and females respectively. In other immature and adult animals, the tumors grew only temporarily and regressed within 15 to 35 days following inoculation.

The results with immature suckling mice have been divided into 2 groups: those obtained in animals

[1] All animals were of the ES strain of albino mice (5). This strain is probably identical with the so-called "Bagg albino" strain (1, 16), having been obtained from the same source and bred continuously by brother-to-sister mating.

[2] In order to demonstrate the effects of castration, tumor inoculation should be delayed, a point already stressed in previous studies (15, 8, 12) and confirmed in our experiments.

[3] Unless otherwise stated in the tables, animals referred to as adults were males and virgin females approximately 3 months of age.

[4] Histologically, these tumors were typical sarcomas.

TABLE I: THE EFFECTS OF CASTRATION ON REGRESSION OF INTRACUTANEOUS SARCOMAS IN ADULT MALE AND FEMALE MICE

			Castrated mice				Normal mice (controls)			
Experiment number	Age at castration, days	Interval between castration and inoculation, weeks	Sex	Number of mice with tumors	Number of mice recovered	Per cent recovered	Sex	Number of mice with tumors	Number of mice recovered	Per cent recovered
1	21–28	6–7	M	17	6	35	M	21	1	5
			F	22	5	23	F	21	6	29
2	21–28	8	M	17	5	29	M	20	6	30
			F	18	3	17	F	17	6	35
3	21–28	10	M	26	7	27	M	20	3	15
			F	28	3	11	..	..	..	..
4	50–60	5	M	20	5	25	M	20	5	25
			F	21	6	29	F	18	8	44
5	50–60	5–6	M	14	1	7	M	19	1	5
			F	13	1	8	F	20	8	40
6	50–60	6	M	19	4	21	M	18	1	6
			F	20	9	45	F	13	8	61
7	50–60	7	M	20	8	40	M	21	2	10
			F	21	6	29	F	20	9	45
8	50–60	8	M	19	6	32	M	21	4	19
			F	20	4	20	F	21	9	43
9	50–60	10	M	20	4	20	M	19	2	11
			F	23	7	30	..	..	..	..
Total, experiments 1–9.....			M	172	46	27	M	179	25	14
			F	186	44	24	F	130	54	42

TABLE II: INCIDENCE OF REGRESSION OF INTRACUTANEOUS TUMORS IN IMMATURE SUCKLING MICE INOCULATED AT THE AGE OF 0 TO 4 DAYS

	Immature suckling mice				Adult controls			
Age at inoculation, days	Sex	Number of mice with tumors	Number of mice recovered	Per cent recovered	Sex	Number of mice with tumors	Number of mice recovered	Per cent recovered
0–4	M	12	2		M	21	3	
	F	22	2		F	20	10	
0–4	M	14	5		No control			
	F	7	4					
0–4	M	14	4					
	F	10	5					
1–4	M	5	2					
	F	4	1					
2–4	M	8	2		M	19	2	
	F	8	2		F	18	3	
2–3	M	9	3		M	21	1	
	F	5	0		F	21	6	
2–3	M	13	5		M	20	4	
	F	15	3		F	18	9	
3–4	M	6	2		M	22	4	
	F	7	0		F	20	9	
Total.....	M	81	25	31	M	103	14	14
	F	78	17	22	F	97	37	38

inoculated within 4 days of birth (Table II) and those obtained in animals inoculated at the ages of 5 to 18 days (Table III). It is evident from the data in these tables that the incidence of regression was the same, within limits of error, in the immature suckling male and female mice. This result was in striking contrast to that obtained with the adult control animals, where more females than males recovered from tumors.

It is noteworthy that the average incidence of regression in mice inoculated at the age of 0 to 4 days was lower than that in the animals inoculated at the age of 5 to 18 days. The average survival time in all fatal cases was 17.2 days for the 0- to 4-day old group as compared with 23 days for the 5- to 18-day old group. Most of the tumors grew rapidly during the first 14 to 16 days following inoculation; many of the 0- to 4-day old animals did not survive this apparently critical period, dying before they had had an opportunity to recover from the relatively large neoplasms. It seems probable that the trauma incident to inoculation and malnutrition as a result of tumor growth had much to do with the death of these very young animals.

TABLE III: INCIDENCE OF REGRESSION OF INTRACUTANEOUS TUMORS IN IMMATURE SUCKLING MICE INOCULATED AT THE AGE OF 5 TO 18 DAYS

	Immature mice				Adult controls			
Age at inoculation, days	Sex	Number of mice with tumors	Number of mice recovered	Per cent recovered	Sex	Number of mice with tumors	Number of mice recovered	Per cent recovered
5–8	M	11	3		M	21	1	
	F	2	0		F	21	6	
5	M	10	2		No control			
	F	8	2					
5–9	M	21	5		M	20	6	
	F	21	4		F	18	7	
5–9	M	12	8		M	13	0	
	F	11	5		F	15	6	
5–11	M	19	11		No control			
	F	19	7					
6–7	M	8	5		M	9	1	
	F	2	0		F	8	1	
6–7	M	9	4		M	19	2	
	F	8	5		F	18	3	
6–8	M	26	9		M	19	1	
	F	22	8		F	20	8	
7	M	3	2		M	18	1	
	..	..	..		F	13	8	
7	M	6	4		No control			
	F	1	0					
7–9	M	14	9					
	F	15	8					
7–11	M	17	7		M	21	2	
	F	17	7		F	20	9	
14	M	1	1					
	F	1	1					
13–18	M	8	3		M	20	5	
	F	12	8		F	18	8	
Total.....	M	165	73	44	M	160	19	12
	F	139	55	40	F	151	56	37

THE RESISTANCE OF IMMATURE WEANED ANIMALS

Immature[5] suckling mice 16 to 26 days old were weaned and inoculated intradermally with sarcoma 37, either on the day of weaning or 1 to 2 days thereafter. Adult mice of both sexes were inoculated simultaneously as controls. All immature and control animals developed tumors within 10 days following inoculation.

The results of these experiments, recorded in Table IV, show that the tumors regressed spon-

[5] Sexual maturity is reached by these mice at the age of 40 to 45 days (5).

taneously in only 12 and 14 per cent of the weaned immature males and females. Thus it is evident that the incidence of tumor regression in the weaned mice was much lower than that in suckling animals 5 to 18 days old (*cf.* data in Tables III and IV).

DISCUSSION

There have been numerous studies on the influence of castration on the resistance of mice and rats to implanted tumors. Some investigators (12, 14, 17) have observed increased resistance in castrated males. Others (7, 10, 11) have noted increased resistance in castrated females; while still others (8) have observed increased resistance in both castrated males and females. On the other hand, certain investigators have reported that the resistance to tumors decreased following castration of males (7, 13, 18, 19) or females (9). A few investigators have observed no effect of castration (2, 3).

These highly conflicting results, representing all possible variations, may be at least partially explained by the methods used in transplanting the tumor and determining the resistance of the host. In some reports, neither the route of inoculation nor the method of tumor implantation was described. In most instances, however, small pieces of tumor were inserted subcutaneously with a trocar, a procedure which practically excludes the possibility of careful dosage. In almost all the previous experiments, the resistance of the castrated animals to the tumor was determined by the incidence of takes or the size of the growing tumors. Our studies (4, 6) have shown, however, that the incidence of takes depends upon the dose of tumor suspension inoculated. Thus without careful dosage, differences in the incidence of takes may be only accidental. Measurements of the size of growing tumors are not a dependable indication of the resistance of the host, since it is well known that fatal metastases may occur in animals bearing only medium sized or small tumors.

The present study on the effects of castration on resistance to the implantation of a tumor differs from those performed previously in 4 respects: in the first place, a carefully measured, standard dose of tumor cell suspension was used throughout the experiments; secondly, all inoculations were made intradermally; thirdly, cutaneous tumors were produced in *all* animals; and lastly, the criterion of the resistance of the host was the incidence of spontaneous and complete recovery of the animals from the cutaneous tumors. Control of these factors has made it possible to demonstrate that castration either before or shortly after the onset of sexual maturity increased the resistance of males to subsequent intradermal inoculation of sarcoma 37, and decreased the resistance of females.

The present experiments have shown also that immature suckling male and female mice, inoculated at the age of 5 to 18 days, have a high resistance to intradermal implantation of sarcoma 37; this resistance is essentially identical with that of young sexually mature females. After weaning, the resistance of immature males and females diminishes rapidly. As the female mouse matures sexually, a high degree of resistance to this sarcoma is again acquired. No such change occurs in the maturing male (4, 5), the resistance of sexually mature male mice being virtually the same as that of recently weaned animals.

TABLE IV: INCIDENCE OF REGRESSION OF INTRACUTANEOUS TUMORS IN IMMATURE WEANED MICE INOCULATED AT THE AGE OF 16 TO 28 DAYS

		Immature weaned mice				Control adults			
Experiment number	Age at inoculation, days	Sex	Number of mice with tumors	Number of mice recovered	Per cent recovered	Sex	Number of mice with tumors	Number of mice recovered	Per cent recovered
1	16–20	M	22	7	32	M	20	4	20
		F	23	5	22	F	18	9	50
2	16–21	M	25	3	12	M	22	4	18
		F	25	4	16	F	20	9	45
3	17–20	M	27	2	7	M	21	2	10
		F	16	1	6	F	20	9	45
4	21–28	M	23	0	0	The same control animals as for experiment 1			
		F	18	2	11				
5	21–28	M	16	2	12	M	18	1	6
		F	15	2	13	F	13	8	60
6	21–28	M	24	2	8	M	20	2	10
		F	19	2	10	F	20	5	25
Total, experiments 1 to 6		M	137	16	12	M	101	13	13
		F	116	16	14	F	91	40	44

The changes in the resistance of female mice from weaning through sexual maturity, correlated with the effects of castration on female animals described above, suggest that the actively functioning ovary in the young adult female mouse directly or indirectly increases resistance to the implantation of sarcoma.

The high degree of resistance of suckling immature male and female mice suggests that these animals have acquired some of the resistance of the adult mother. The fact that this resistance is lost promptly after weaning suggests that the resistance factor may have been transmitted through the milk; the possibility, however, of placental transmission of some resistance factor must also be considered. Further studies are necessary to determine which, if either, of these possibilities is responsible for the high resistance of suckling, and the low resistance of weaned immature animals.

SUMMARY AND CONCLUSIONS

The resistance of castrated and of sexually immature suckling and weaned mice of the albino ES strain to sarcoma 37 was determined by the incidence of spontaneous regression of tumors produced by intradermal inoculation of a standard dose of tumor cell suspension.

Castration either before or shortly after the onset of sexual maturity increases the resistance of males to subsequent intradermal inoculation of sarcoma and decreases that of females.

Immature suckling male and female mice, inoculated at the age of 5 to 18 days, have a high resistance to sarcoma not unlike that of adult females.

Sexually immature males and females, inoculated at the age of 16 to 26 days, immediately or shortly after weaning, have a low resistance to sarcoma not unlike that of adult males.

Since the weaned female mouse again acquires a high degree of resistance to sarcoma after sexual maturity (4, 5), whereas no such change occurs in the maturing male (4, 5), it may be concluded that the actively functioning ovary in the young adult female mouse directly or indirectly increases the resistance of the host to the intradermal implantation of sarcoma. This conclusion is supported further by the findings on castrated female mice.

The observation that the resistance of immature suckling mice is lost promptly after weaning suggests maternal transmission of a resistance factor either through the milk or through the placenta.

In conclusion it may be stated that the striking difference in the incidence of spontaneous regression of intracutaneous tumors in adult male and female mice (4, 5) cannot be demonstrated either in castrated or in sexually immature suckling or weaned animals.

REFERENCES

1. Bagg, H. J. The Role of Functional Activity in the Production of Mammary Carcinoma. Am. Naturalist, **60**: 234-239. 1926.
2. Bischoff, F., Maxwell, L. C., and Ulmann, H. J. Hormones in Cancer. III. Effect of Glandular Extirpation. J. Biol. Chem., **92**:lxxx. 1931.
3. Graf, R. Versuche über das Wachstum von Tumoren nach Kastration. Centralbl. f. allg. Path. u. path. Anat., **20**: 783-786. 1909.
4. Gross, L. Influence of Sex on the Evolution of a Transplantable Mouse Sarcoma. Proc. Soc. Exper. Biol. & Med., **47**:273-276. 1941.
5. Gross, L. Influence of Sex of Mice on Acquired Resistance to a Transplantable Sarcoma. Cancer Research, **1**:880-882. 1941.
6. Gross, L. Influence of Sex on Resistance to Intraperitoneal Inoculation of Sarcoma in Mice. Proc. Soc. Exper. Biol. & Med., **49**:67-71. 1942.
7. Heiman, J., and Krehbiel, O. F. The Influence of Hormones on Breast Hyperplasia and Tumor Growths in White Rats. Am. J. Cancer, **27**:450-473. 1936.
8. Murphy, Jas. B., and Sturm, E. Effect of Prepuberty Castration on Subsequent Cancer Implantation. J. Exper. Med., **42**:155-161. 1925.
9. Nathanson, I. T., and Salter, W. T. Experimentally Induced Benignancy of Neoplasm. II. The Effect of Treatment with an Estrogen and of Castration of the Host. Arch. Path., **27**:828-840. 1939.
10. Nitta, Y. Female Sexual Hormones and Malignant Tumors. I. Effect of Castration on Malignant Tumors. II. Effect of Ovarian Hormones on Malignant Tumors. Jap. J. Obst. & Gynec., **19**:90-109. 1936; abstr., Am. J. Cancer, **28**:783. 1936.
11. Pribram, E. Studien zur Geschwulstimmunität. XII. Ovarialfunktion und Tumorwachstum bei der weissen Maus. Ztschr. f. Krebsforsch., **34**:545-550. 1931.
12. Pribram, E. Studien zur Geschwulstimmunität. XV. Weitere Versuche über die Einwirking der männlichen Keimdrüsen auf Angehen und Wachstum von Transplantationstumoren. Ztschr. f. Krebsforsch., **42**:368-372. 1935.
13. Reiss, M., Druckrey, H., and Hochwald, A. Tumor und Inkretsystem. Klin. Wchnschr., **12**:1049-1050. 1933.
14. Rohdenburg, G. L., Bullock, F. D., and Johnston, P. J. The Effects of Certain Internal Secretions on Malignant Tumors. Arch. Int. Med., **7**:491-499. 1911.
15. Strong, L. C. Indications of Tissue Specificity in a Transplantable Sarcoma. J. Exper. Med., **39**:447-456. 1924.
16. Strong, L. C. The Establishment of the "A" Strain of Inbred Mice. J. Hered., **27**:21-24. 1936.
17. Strong, L. C., Hill, R. T., Pfeiffer, C. A., and Gardner, W. U. Genetic and Endocrine Studies on a Transplantable Carcinoma of the Ovary. Genetics, **23**:585-595. 1938.
18. Sweet, J. E., Corson-White, E. P., and Saxon, G. J. The Relation of Diets and of Castration to the Transmissible Tumors of Rats and Mice. J. Biol. Chem., **15**:181-191. 1913.
19. Wagner, A. The Effect of Castration on Resistance to Cancer. Hospitalstid., **75**:817-829, 1932; abstr., Am. J. Cancer, **17**:183. 1933.

Complement-Fixing Antibodies (Brown-Pearce Carcinoma) in the Blood Serum and in the Aqueous Fluid of the Anterior Chamber of the Eye*

Max Appel, M.D., Otto Saphir, M.D., Martha Janota, M.S., and Alfred A. Strauss, M.D.

(From the Departments of Pathology and Bacteriology of the Michael Reese Hospital, Chicago, Ill.)

(Received for publication April 15, 1942)

Intracutaneous transplantation with the Brown-Pearce carcinoma results in a tumor which, after growing to a certain size, regresses, leaving the animal immune to subsequent growth of this tumor (2, 3, 9). We have recently shown (10) that such immunization does not confer protection against growth of this tumor in the anterior chamber of the eye, despite the fact that all other tissues tested are resistant following regression of the skin tumor.

The lack of immunity of the anterior chamber of the eye of the immune rabbit to this tumor may result from the barrier which exists between the blood plasma and the aqueous humor, preventing the passage of antibodies from the former into the latter (5). This may be explained by the fact that the aqueous humor is formed by a process of dialysis from the blood plasma. Crystalloids readily pass into the anterior chamber but larger molecules, the size of serum albumin or larger, are retained. The work of Becht and Greer (1), and also that of Hektoen and Carlson (7), who studied the concentration of antibodies in the various body fluids of animals immunized to bacterial and other foreign proteins, supports this assumption. They found that the titer of immune bodies was highest in the serum, and weakly positive or negative in the aqueous fluid of the anterior chamber of the eye.

Therefore it was thought advisable to study the comparative concentration of tumor antibodies in the blood serum and in the aqueous fluid of the anterior chamber of the eyes of rabbits bearing the Brown-Pearce carcinoma. The presence of tumor antibodies in the serum and their absence from the aqueous fluid in the same animal at the same time would indicate that such a barrier does exist and would provide support for the assumption that the successful growth of the Brown-Pearce carcinoma in the anterior chamber of the eye of the tumor-immune animal is referable to the failure of antibodies to gain access to the aqueous fluid.

To determine the presence or absence of tumor antibodies in these fluids, the complement fixation test as described by Kidd (8) and by Cheever (4) was used. Kidd reported almost 100 per cent positive reactions. We performed complement fixation tests according to Kidd's technic on the serums from rabbits with tumor but did not obtain results as consistently positive as did Kidd. However, repeated tests on the blood serum during the course of the development of the tumor disclosed the presence of complement-fixing antibodies at some stage in its course. The positive complement fixation reaction was not maintained, however. Nevertheless, we decided to use this immune reaction to determine the relative concentrations of antibodies in the blood serum and in the aqueous fluid.

Twenty-four tumor-bearing rabbits, on which complement fixation tests were made at intervals of 1 to 3 days from the time of the appearance of the tumor, were used for this experiment. In all these animals a 3 to 4 plus complement fixation reaction was obtained in the blood serum at some time during the course of the development of the tumor. Though this usually corresponded with the height of development of the tumor, such was not always the case. As soon as complement-fixing antibodies were identified in the blood serum, aqueous fluid was withdrawn from the anterior chamber of the eye. Because of the decided variations in the titer of complement-fixing antibodies in the serum, it was considered essential that they be determined in the aqueous fluid on the same day on which a positive reaction was obtained in the serum. The technic of withdrawing aqueous fluid was as follows: The animals were anesthetized with intravenous nembutal. The eye was grasped

* This investigation was aided by grants from the Woman's Board of the Michael Reese Hospital and the Gusta M. Rothschild Fund.

with a fixation forceps and rotated so that the corneo-limbal junction was readily accessible; then a long hypodermic needle, attached to a 1 cc. tuberculin syringe, was inserted through the corneo-limbal junction into the chamber and fluid withdrawn, about 0.25 to 0.5 cc. from each eye.

The antigen used in the tests was prepared from tumor tissue removed aseptically from stock tumor animals. Only firm cellular portions of tumor were selected, necrotic portions being discarded. Tumor tissue was preserved in 50 per cent Locke-glycerol solution until ready for use. Portions were then mixed with sterile sand and saline and thoroughly ground in a mortar with a pestle. This mixture was allowed to stand in the icebox for 24 hours, centrifuged at 2,000 r.p.m. for 15 minutes, and the supernatant fluid separated and placed in a water bath at 60° C. for 30 minutes. The anticomplementary unit of the antigen thus prepared was determined and the antigen diluted so that 0.2 cc., the amount used in the tests, was equivalent to one-quarter of the anticomplementary unit. The titer of this antigen was then tested with a known positive serum. Only antigen which yielded a 4 plus reaction with a known positive blood serum was used for the tests. The strength of the antigen was found to diminish considerably after standing for 4 to 6 weeks at icebox temperature.

Four tube tests were set up with 0.2 cc. antigen and 0.02, 0.03, and 0.1 cc. of blood serum which had been inactivated for one-half hour at 56° C. One-tenth cc. of 10 per cent guinea pig serum was added to each tube and the mixture placed in a water bath at 37° C. for 1 hour. Two-tenths cc. of a mixture of equal parts of 5 per cent sheep cells and amboceptor (2 units) were added and incubated again at 37° C. for 20 minutes, and the results of the tests noted.

Inasmuch as only relatively small amounts of aqueous humor could be obtained, the technic of the test had to be modified slightly when applied to this fluid. Only one amount of aqueous humor, 0.4 cc., was used in each test; otherwise the procedure was the same as that used for serum.

The results were quite clear-cut and consistent. As previously noted, all the blood serums gave at least on one occasion a 3 to 4 plus positive complement fixation reaction. Aqueous humor obtained from the eyes of these animals, at the height of complement fixation titer in the serum, invariably gave a negative complement fixation reaction. In not a single instance was it possible to demonstrate complement-fixing antibodies in the aqueous humor of tumor-bearing rabbits even though a high titer of such antibodies could be demonstrated at the same time in the blood serum of these animals.

It is known that if the eye is disturbed, so that the intraocular capillaries become dilated, the permeability of these capillaries becomes altered and the composition of the aqueous fluid changes so that it approximates more closely that of the blood plasma, particularly in respect to its protein content. Such an alteration in the permeability of the intraocular capillaries can be brought about by puncture of the anterior chamber and evacuation of the aqueous fluid. The fluid which refills the chamber under these circumstances has a protein content which approximates that of the blood, and apparently antibodies which were formerly not present, or present in very low concentration, now appear in the aqueous humor in definitely increased concentration. Immune bodies in newly formed aqueous fluid may be increased up to 100 times (6).

Accordingly, it was thought advisable to examine the antibody content of the re-formed aqueous fluid in 12 of the animals which had previously yielded negative complement fixation reactions in the aqueous fluid. It was found that in this re-formed aqueous a 1 to 3 plus complement fixation reaction was obtained.

The absence of immune bodies from the aqueous fluid of the eye while present in high titer in the serum of the same animal suggests that a barrier exists which prevents the passage of antibodies from the serum into the aqueous fluid. This provides a possible explanation for the successful growth of the Brown-Pearce carcinoma in the anterior chamber of the eye of the tumor-immune rabbit when the tumor will not grow in any of the other usual sites of transplantation following immunization. The appearance of complement-fixing antibodies in the re-formed aqueous is not surprising. This fact has been known to ophthalmologists for many years. A recognized therapeutic procedure in the treatment of eye infections is to perform an anterior chamber puncture, withdrawing aqueous fluid. Because of the increased antibody content in the re-formed fluid, these patients frequently show distinct improvement.

SUMMARY

Immunization of rabbits against the Brown-Pearce carcinoma does not confer protection against the growth of this tumor in the anterior chamber of the eye. Complement fixation tests made with the blood serum and the aqueous humor reveal that, although complement-fixing antibodies are present in the blood of tumor-bearing and tumor-immune rabbits, they do not gain access to the aqueous fluid. It is suggested that the barrier which exists between the blood and the aqueous humor, preventing the passage of antibodies from the former into the latter, is responsible for the successful growth of the Brown-Pearce carci-

noma in the anterior chamber of the eye of tumor-immune rabbits when it will not grow in any other location.

REFERENCES

1. Becht, F. C., and Greer, J. R. A Study of the Concentration of the Antibodies in the Body Fluids of Normal and Immune Animals. J. Infect. Dis., **7**:127-158. 1910.
2. Besredka, A., and Gross, L. Vaccination du lapin contre l'épithélioma sous-cutané. Compt. rend. Acad. d. sc., **202**:1217-1219. 1936.
3. Besredka, A., Magat, L., Laval, P., and Besnard, P. L'épithélioma intracutané du lapin et son pouvoir immunisant. Ann. Inst. Pasteur, **56**:125-136. 1936.
4. Cheever, F. S. Complement-Fixing Antibody in Sera of Rabbits Bearing Brown-Pearce Carcinoma. Proc. Soc. Exper. Biol. & Med., **45**:517-522. 1940.
5. Davson, H., and Quilliam, J. P. The Permeability of the Blood-Aqueous Humour Barrier to Potassium, Sodium, and Chloride in the Surviving Eye. J. Physiol., **98**:141-154. 1940.
6. Duke-Elder, W. S. Text-Book of Ophthalmology. Vol. II. P. 1453. St. Louis: The C. V. Mosby Company. 1938.
7. Hektoen, L., and Carlson, A. J. On the Distribution of Antibodies and Their Formation by the Blood. J. Infect. Dis., **7**:319-333. 1910.
8. Kidd, J. G. A Distinctive Substance Associated with the Brown-Pearce Rabbit Carcinoma. II. Properties of the Substance: Discussion. J. Exper. Med., **71**:351-371. 1940.
9. Pearce, L., and Brown, W. H. Studies Based on a Malignant Tumor of the Rabbit. IV. The Results of Miscellaneous Methods of Transplantation, with a Discussion of Factors Influencing Transplantation in General. J. Exper. Med., **37**:811-828. 1923.
10. Saphir, O., Appel, M., and Strauss, A. A. Growth of Brown-Pearce Carcinoma in the Anterior Chamber of the Eyes of Tumor-Immune Rabbits. Cancer Research, **1**:545-547. 1941.

Abstracts

Reports of Experimental Research

Carcinogenic Compounds

CAMPBELL, J. A. [National Inst. for Med. Research, London] LUNG TUMOURS IN MICE. INCIDENCE AS AFFECTED BY INHALATION OF CERTAIN CARCINOGENIC AGENTS AND SOME DUSTS. Brit. M. J., 1:217-221. 1942.

The author has continued his investigations on induced lung tumors (see Cancer Research, 1:328. 1941) by exposing 453 mice to 6 dusts; namely, (a) silica + methylcholanthrene, (b) steel grindings, (c) alumina + silica + brown oxide of iron, (d) Joachimsthal mine dust, (e) same as (c) + calcium carbonate, (f) dust of a road tarred 5 years ago, containing 2% of tar. The percentage incidence of lung tumors in mice which survived 10 months of exposure to (a) was 23.8, to (b) 13.6, (c) 15.8, (d) 20.3, (e) 19.4, (f) 13.1; mean 17.7. Seventy-five mice were exposed to each dust and the same number of mice were associated with each series as controls; the percentage incidence of lung tumors in these control series was 13.6, 7.7, 9.2, 2.1, 0.0, 1.4; mean 5.8, or about one-third of that in the series exposed to dusts. Thus all 6 dusts, whether these did or did not contain any constituent shown by other methods to be carcinogenic, increased the incidence of lung tumors. The addition of methylcholanthrene to silica produced an incidence of tumors not greater than that which would be expected from silica alone. In all, there were lung tumors in 21 control and 65 dusted mice; the tumors in 10 of the former, and in 30 of the latter were malignant; *i.e.*, about one-half were malignant in both series. Of these tumors 64% were in females and 36% in males (at the beginning of the experiment the numbers of the sexes were equal; the comparative survival rate is not stated). "There was nearly a fourfold increase in the incidence of hyperplasia of lymph tissue of the lung and a threefold increase for the tracheo-bronchial lymph glands as the result of the dusting. The types of cells concerned in this reticulosis were sometimes the lymphocytes chiefly and sometimes the reticulum cells, and again sometimes both more or less equally." The stock of mice (white, fawn, and chocolate, not pure line) used by the author has recently shown a diminution in the incidence of spontaneous tumors, nearly all of the lung, from 12 to 7%, and the tumors of the lung are now smaller in size. Lung tumors are very rare before the 10th month of life. The author does not give data upon the numbers of lung tumors in individual mice. The paper deals also with the development of silicotic nodules.—E. L.K.

FIESER, L. F., and HEYMANN, H. [Harvard Univ., Cambridge, Mass.] SYNTHESIS OF 2-HYDROXY-3,4-BENZPYRENE and 2-METHYL-3,4-BENZPYRENE. J. Am. Chem. Soc., 63:2333-2340. 1941.

It is noted that 9-methyl-3,4-benzpyrene produces tumors in mice in 12 weeks and thus is at least as potent a carcinogen as 3,4-benzpyrene and 5-methyl-3,4-benzpyrene. The 6-methyl-3,4-benzpyrene is distinctly less active than the above mentioned isomers.—H. J. C.

HERSHBERG, E. B., and FIESER, L. F. [Harvard Univ., Cambridge, Mass.] ISOLOGS OF 9,10-DIMETHYL-1,2-BENZANTHRACENE CONTAINING SULFUR AND SELENIUM. J. Am. Chem. Soc., 63:2561-2564. 1941.

The thiophene isolog of 9,10-dimethyl-1,2-benzanthracene (J. Am. Chem. Soc., **62**:3098. 1940) produced tumors in mice in an average time of 18 weeks. The importance of this observation has led to the syntheses of other isologs containing sulfur and selenium. The syntheses have been developed with the purpose of adaptation to the introduction of radioactive sulfur as a tracer element.—H. J. C.

LIU, Y., and HU, C. H. [Peiping Union Med. Coll., Peiping, China] EXPERIMENTAL PRODUCTION OF TUMORS BY TARS FROM VARIOUS FOODS. Proc. Soc. Exper. Biol. & Med., 48:226-227. 1941.

Polished rice, pea flour, soybean, casein, wheat gluten, whole wheat, corn, cabbage, pepper, beef, and pork were each, in dry form, heated to effect destructive distillation. The water in the distillate was removed with chloroform. The water and chloroform fractions were then separated. The chloroform was removed from its fraction by distillation, and the water fraction concentrated on a steam bath. These final fractions were then combined and used for painting.

Eleven groups of white mice 3 to 4 months old, 10 mice per group, were used. On alternate days, the depilated skin of the back at the scapulae was painted, one group for each kind of tar. Five animals painted with carcinogenic coal tar served as controls.

Up to the time of writing, 230 mice were painted from 15 to 343 days. Of these, 4 developed papillomas (wheat gluten 1; pepper 1; cabbage 2) in 52 to 315 days. The 5 controls developed 3 papillomas and 2 carcinomas in 60 to 83 days.—M. B.

McDONALD, S., Jr., and WOODHOUSE, D. L. [Birmingham Univ., Birmingham, England] ON THE NATURE OF MOUSE LUNG ADENOMATA, WITH SPECIAL REFERENCE TO THE EFFECTS OF ATMOSPHERIC DUST ON THE INCIDENCE OF THESE TUMOURS. J. Path. & Bact., 54:1-12. 1942.

In histological study of lung tumors in 71 mice of which 69 bore the Tyzzer type of adenoma, one, in which a dibenzanthracene sarcoma had been grafted in the leg,

Microfilm copies of such papers here abstracted as are available may be obtained from Medicofilm Service of the Army Medical Library at 25¢ for each complete article, not exceeding 25 pages in length—and 10¢ for each additional 10 pages or fraction thereof. Prepayment is not requested. Remittance may be made with subsequent orders and in such manner as found most convenient. Address—Medicofilm Service, Army Medical Library, Washington, D. C.

showed a primary adenocarcinoma with multiple simple adenomata, and one, painted with tobacco tar, showed carcinomatous transformation of adenoma. In another mouse painted with tobacco tar there was diffuse adenomatous transformation of one lung and over 20 adenomata in the other lung. In the whole series, 67% bore single and 33% bore multiple adenomas, and in a few "the entire lung appeared to have undergone adenomatous transformation." Mice were exposed (a) to dust collected in Birmingham from the air filters of a plenum system ("plenum dust"), (b) to dust from roofs, and (c) to zinc dust; and treated in other ways: (d) grafting of a dibenzanthracene sarcoma, and application to the skin of (e) tobacco tar (carcinogenic to the skin), (f) bacterial decomposition products of meat, and (g) solutions of deoxycholic acid and egg albumin irradiated with ultraviolet, and (h) extract of plenum dust. The percentage incidence of pulmonary adenoma in these series was (a) 22.8, (b) 4.8, (c) 14.3, (d) 2.6, (e) 19.0, (f) 19.3, (g) 5.8, and (h) 25.0. This percentage is greater than that in controls (15.8) only in (a), (e), (f), and (h).

Spectroscopic comparison of an extract of plenum dust, and of a solution of 3,4-benzpyrene, indicated that this compound was present in the dust. No such examination of roof dust is mentioned.

The paper consists partly of abstracts of the papers of earlier workers on this subject. The authors refer to the well known resemblance of the lung adenoma of mice to 2 conditions of the lung in sheep, namely the jagziekte in South Africa and the adenomatosis in Iceland described by Dungal (Proc. Roy. Soc. Med., **31**:497. 1937-38); 2 microphotographs of Dungal's preparations are given. The authors come finally to the conclusion that the Tyzzer adenoma is not "a true malignant neoplasm" though they do not say who holds any such view. The adenoma spreads by progressive metaplasia of the alveolar epithelium at the periphery; intrabronchial papillary hyperplasia is frequent also. Mitotic activity was not seen. There is a uniformly distributed elastic stroma. Destructive invasion of the lung is not evident.—E. L.K.

PENN, H. S. [Los Angeles, Calif.] SPECTRA OF LIPOID FRACTIONS FROM HUMAN NON-CANCEROUS AND CANCEROUS TISSUE. J. Chem. Phys., 10:145-146. 1942.

While the fluorescence spectra of lipoid fractions from liver of cancerous and control subjects lay in the same general region, that from the latter was considerably weaker. With increasing deproteinization of the tissue associated with cancer the strong fluorescence excited with the light of a mercury arc approached the characteristic spectrum of methylcholanthrene. The author relates these findings to the possibility that cancer may be caused by carcinogenic substances developed in the body. —M. J. E.

1. PENN, H. S. [Univ. of California, Los Angeles, Calif.] FLUORESCENT LIPOIDAL SPECTRA OF HUMAN TISSUE Nature, London, 149: 193-194. 1942.

2. HIEGER, I. [Chester Beatty Research Inst., Royal Cancer Hosp. (Free), London] FLUORESCENCE OF METHYLCHOLANTHRENE. Nature, London, 149:300-301. 1942.

1. (a) "Hieger pointed out that most of the carcinogenic hydrocarbons studied were highly fluorescent and they produced characteristic bands in the regions 4,000, 4,180, and 4,400 A." (b) Cancer tissue lipoids produce a far stronger fluorescence than do those of nonmalignant tissue. (c) "Spectrographic characteristics identical with those found in methylcholanthrene are evident in the lipoid of human cancer tissue (carcinoma)." Photographs of the fluorescence spectra of cancerous and noncancerous tissue, and of methylcholanthrene, are given.

2. The writer comments on the letter above to the effect that (a) is incorrect as the spectrum in question was observed only in complex carcinogenic mixtures containing 3,4-benzpyrene; that his own observations do not confirm (b); and that the spectrum shown is not that of methylcholanthrene.—E. L. K.

STEINER, P. E. [Univ. of Chicago, Chicago, Ill.] EPITHELIAL HYPERPLASIA OF HASSALL'S BODIES OF THYMUS GLAND INDUCED BY METHYLCHOLANTHRENE. Proc. Soc. Exper. Biol. & Med., 49:62-67. 1942.

Cylindrical pellets, composed of 10 mgm. methylcholanthrene plus 10 mgm. cholesterol, about 1.75 mm. in diameter and 8 to 10 mm. in length were implanted into the thymus gland of young guinea pigs of both sexes. Animals were sacrificed at 7, 14, 21, and 28 days and at other intervals. Sections were made with a variety of fixatives and stains.

The hyperplasia of Hassall's bodies in the region of the pellet was seen as early as the 7th day. At 14 days, and from then on, squamous epithelialization of the pellet space, epithelial cysts, and degeneration of the small thymocytes were observed. These new epithelia showed intercellular bridges and keratohyaline formation and, as they arose from Hassall's bodies, indicated the epithelial nature of the letter.

Control cholesteral pellets, implanted in the thymus and in other tissues and organs located in the neck did not show similar changes.—M. B.

SUGIURA, K., and RHOADS, C. P. [Memorial Hosp., New York, N. Y.] THE EFFECT OF YEAST FEEDING UPON EXPERIMENTALLY PRODUCED LIVER CANCER AND CIRRHOSIS. Cancer Research, 2:453-459. 1942.

1. The therapeutic action of yeast upon liver cirrhosis and cancer has been investigated. The liver changes were induced in rats by feeding unpolished rice and *p*-dimethylaminoazobenzene.

2. Cirrhosis was absent in the livers of rats if butter yellow had been given for less than 32 days. However, if the preliminary feeding with butter yellow exceeded 60 days, liver cancers developed in a large percentage of animals surviving on a rice diet fed subsequent to the butter yellow-rice diet for over 100 days. The longer the period on the butter yellow-rice diet, the greater the incidence of resultant liver cancer.

3. Liver cirrhosis produced by butter yellow has been treated successfully by a rice diet containing 15% yeast.

4. Once adenomatous hyperplasia of bile ducts, cholangioma, or hepatoma had been established in the liver, these benign and malignant tumors could not be destroyed by ingestion of the rice-yeast diet.—Authors' summary.

Hormones

BISCHOFF, F., LONG, M. L., RUPP, J. J., and CLARKE, G. J. [Santa Barbara Cottage Hosp. Research Inst., Santa Barbara, Calif.] ENDOCRINE FACTORS INFLUENCING

TUMOR DEVELOPMENT. THE EFFECT OF THE GONADOTROPINS AND OF THEELIN UPON THE MARSH-BUFFALO ADENOCARCINOMA AND LYMPHOSARCOMA. Endocrinology, 28:769-779. 1941.

Parenteral administration of prolan, pregnant mare serum, or pituitary gonadotropins in large doses and intermittent treatment with these hormones over prolonged periods, accelerated the acinar development of the mammary glands in young mice of the Marsh-Buffalo strain, but did not increase the incidence of adenocarcinoma of the breast. Pregnant mare serum and pituitary gonadotropins significantly retarded the onset and decreased the incidence of this tumor. When the incidence of adenocarcinoma was decreased, that of lymphosarcoma increased. Intermittent treatment for a year with gonadotropins did not prevent pregnancy. Even sublethal doses of theelin (3.8 mgm. per mouse in 5 months) failed to accelerate acinar development of the mammary gland but gave a doubtfully significant increase in adenosarcomas and lymphosarcomas. The Marsh-Buffalo mice are susceptible to cancer and resistant to theelin when compared with other high cancer strains of mice.—C. A. F.

GARDNER, W. U. [Yale Univ. Sch. of Med., New Haven, Conn.] PERSISTENCE AND GROWTH OF SPONTANEOUS MAMMARY TUMORS AND HYPERPLASTIC NODULES IN HYPOPHYSECTOMIZED MICE. Cancer Research, 2:476-488. 1942.

Thirty-four first generation hybrid mice bearing spontaneous mammary tumors were hypophysectomized either during the latter half of pregnancy or postpartum. The body weights of most of the pregnant mice increased as much as those of the pregnant nonhypophysectomized controls. The mice were killed at periods up to 56 days after operation. In the hypophysectomized mice, the mammary cancers in most cases grew progressively and new tumors appeared frequently; localized hyperplastic nodules ("precancerous") were found in the otherwise atrophic mammary glands; the adrenal cortices decreased in thickness and the cortical cells in size. The mammary glands of the 13 nonhypophysectomized control mice may have contained more hyperplastic nodules. The microscopic structure of the tumors and nodules of the experimental and control mice were similar.

The progressive growth of spontaneous mammary adenocarcinomas and of "precancerous" localized hyperplastic nodules occurred in the absence of the hypophysis.—Author's abstract.

GESCHICKTER, C. F., and BYRNES, E. W. [Baltimore, Md.] FACTORS INFLUENCING THE DEVELOPMENT AND TIME OF APPEARANCE OF MAMMARY CANCER IN THE RAT IN RESPONSE TO ESTROGEN. Arch. Path., 33: 334-356. 1942.

All the estrogens of sufficient potency for clinical use, regardless of chemical composition or physiologic potency, will produce mammary cancer in the rat. To produce mammary cancer the dose must be well beyond the physiologic limit and the treatment continuously applied for a period of months. The total dose required is not influenced by the amount of daily dose but varies with the duration of estrogenic activity and the method of administration. It is difficult to demonstrate that sex, age, or castration influences susceptibility, but there are important species differences. A case of estrogenic mammary cancer in the rabbit is recorded. Administration of testosterone or progesterone does not prevent the appearance of mammary cancer but it is inhibited by anterior pituitary extract. Various changes in the endocrine glands accompany the appearance of mammary cancer, and cancerous change has been observed in other organs. Estrogens exert physiologic rather than direct chemical action, for the lesion does not appear at the site of injection but in the breast, and the time required to produce cancer is related to the physiologic potency and not to the chemical formula.—H. G. W.

Viruses

BEARD, J. W., TAYLOR, A. R., SHARP, D. G., and BEARD, D. [Duke Univ. Sch. of Med., Durham, N. C.] THE NATURE OF A VIRUS ASSOCIATED WITH CARCINOMA IN RABBITS. Surg., Gynec. & Obst., 74:509-513. 1942.

The specific material from virus-induced papillomas of the cottontail rabbit, which behaves biologically as the virus responsible for the disease, is a nucleoprotein obtainable in solutions of a high degree of homogeneity. Its physical properties with respect to sedimentation, electrophoresis, diffusion, and viscosity are those of a molecular substance with values indicating a molecular weight of 47,100,000. With respect to the physical state, this animal virus falls into the same category as that of the macromolecular plant virus nucleoproteins. The nucleic acid of the nucleoprotein is definitely of the thymus type, there being no detectable trace of ribose or yeast nucleic acid.—H. G. W.

DANNEEL, R. [Kaiser Wilhelm Inst., Berlin-Dahlem, Germany] EIN PAPILLOM-VIRUS AUS KANINCHENHAUT. [A PAPILLOMA VIRUS OBTAINED FROM RABBIT SKIN.] Naturwissenschaften, 29: 364-365. 1941.

Extracts of the normal skin of 4 out of 5 untreated domestic rabbits possessed the capacity of inducing papillomas when applied to the abraded skin of other animals, and in one instance it was possible to produce an autoinfection. The lesions closely resembled those resulting from the action of the Shope virus and were transmissible by cell-free extracts to other domestic rabbits.

The possibility that the etiologic agent of all rabbit papillomas is a normal inhabitant of the skin is suggested.—M. J. E.

HALBERSTAEDTER, L., DOLJANSKI, L., and TENENBAUM, E. [Hebrew Univ., Jerusalem, Palestine] EXPERIMENTS ON THE CANCERIZATION OF CELLS IN VITRO BY MEANS OF ROUS SARCOMA AGENT. Brit. J. Exper. Path., 22:179-187. 1941.

By subjecting tissue cultures of a Rous sarcoma to appropriate doses of radium irradiation it is possible to kill the cells without destroying the activity of the "sarcoma agent." The latter is inactivated when cultures are incubated for 48 hours immediately after irradiation, unless living cells are introduced. The introduced cells become infected with the agent and acquire, in the course of several passages, all the morphological and biological characteristics of the malignant cells of this sarcoma. That such cells retain their malignancy was demonstrated by inoculating cultures into chicks after varying periods of cultivation.—R. J. L.

SHEMIN, D., and SPROUL, E. E. [Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.] STUDIES OF THE TRANSMISSIBLE AGENT OF CHICKEN SARCOMA I. ISOLATION OF VIRUS FROM BASIC PROTEIN-VIRUS COMPLEX. Cancer Research, 2:514-516. 1942.

On the addition of a basic protein, papain, to extracts of chicken sarcoma I a precipitate formed which contained the virus. This complex between virus and papain was dissociated by means of sodium chloride. Separation of the virus from the basic protein was then possible by taking advantage of the high molecular weight of the virus. On high speed centrifugation of the dissociated complex, the active fraction was deposited free of papain and electrophoretically homogeneous at pH 7.4.—Authors' abstract.

Biochemistry and Nutrition

GOLDSTEIN, B. J., VOLKENSON, D. V., and KACHEROVA, S. A. [Kiev Roentgeno-Radiological Inst. and Biochemical Inst. of Academy of Science, Ukraine S. S. R.] ON THE FORMS OF ASCORBIC ACID IN TISSUES. Biochem. J. Ukrainian S. S. R., 17:216-217. 1941.

Dehydroascorbic acid is practically absent from most rat tissues (liver, spleen, kidneys, Jensen sarcoma) but occurs in small amounts in the lungs. Bound ascorbic acid may be determined by extraction with 3% metaphosphoric acid with simultaneous reduction or by reduction of protein-free filtrates with nascent hydrogen. The content of bound ascorbic acid parallels the masked iron content and, in tumors, where masked iron is absent, bound ascorbic acid is not found. In liver and spleen the bound ascorbic acid is nearly equal to the amount of free ascorbic acid.

The bound ascorbic acid seems to consist of a combination of ascorbic acid, protein, and masked iron. Possibly 2 forms occur which differ in their extractability by metaphosphoric acid.—F. L. W.

KUBOWITZ, F., and OTT, P. [Kaiser Wilhelm Inst., Berlin-Dahlem, Germany] ISOLIERUNG UND KRISTALLISATION EINES GÄRUNGSFERMENTS AUS TUMOREN. [ISOLATION AND CRYSTALLIZATION OF A RESPIRATORY ENZYME FROM TUMORS.] Naturwissenschaften, 29:590-591. 1941.

An enzyme acting on phosphopyruvic acid to form lactic acid was isolated in crystalline form as a mercury salt from the dried healthy portion of Jensen rat sarcoma. A comparable substance was extracted from muscle tissue, but determinations of its activity had not progressed sufficiently to test its relationship to the tumor enzyme.—M. J. E.

LASNITZKI, A., and BREWER, A. K. [Med. Sch., University of Birmingham, England, and U. S. Dept. of Agriculture, Washington, D. C.] AN ISOTOPIC SHIFT OF POTASSIUM IN HUMAN BONE-MARROW AND CANCER. Nature, 149: 357-358. 1942.

Previous work (*cf.* Cancer Research, 1:776-778. 1941) on the isotopic constitution of potassium in bone marrow and tumor tissue of the rat was extended to the examination of human tissues. The normal noncancerous tissues included 5 samples of bone marrow from lumbar vertebrae and 15 samples from liver, kidney, lung, spleen, brain, heart, and skeletal muscles. Cancerous tissue included 8 samples of primary carcinomas originating in liver, kidney, lung, stomach, colon, and rectum, and 3 samples of liver metastases.

The 5 samples of bone marrow gave values for the $^{41}_{39}$K ratio in excess of the ratio for mineral postassium. Nine of the 15 normal tissues were in close agreement with this ratio, while 6 were slightly lower. All the 11 values for cancer tissue were lower than any of those found for normal tissues. In general the results agree with those obtained with corresponding tissues of nonhuman origin.—F. L. W.

LASNITZKI, A., and BREWER, A. K. [Univ. of Manchester, England, and U. S. Dept. of Agriculture, Washington, D. C.] THE ISOTOPIC CONSTITUTION OF POTASSIUM IN NORMAL TISSUE AND CANCER FROM HUMAN SUBJECTS. Cancer Research, 2:494-496. 1942.

The isotopic constitution of potassium in human cancer has been studied and compared with that of potassium in various normal human tissues. An appreciable difference in the isotopic ratio K^{39}/K^{41}, and consequently in the percentage of the heavy isotope K^{41}, was observed. The difference agreed, both in direction and magnitude, with that previously obtained with potassium in normal tissues and tumors from animals.—Authors' summary.

MINOR, A. H., and RAMIREZ, M. A. [City Hosp., Welfare Island, New York, N. Y.] THE UTILIZATION OF VITAMIN C BY CANCER PATIENTS. Cancer Research, 2:509-513. 1942.

Thirteen hospital patients (7 controls, 1 with localized cancer and 5 with metastatic cancer) were injected daily for several weeks with 500 mgm. of vitamin C, and the plasma and urinary responses noted. After tissue saturation had been attained, as shown by a relatively constant excretion of the vitamin at a high level, the daily utilization was computed as 500 mgm. less than 24 hour urine values. The daily utilization of vitamin C averaged 67 mgm. for the noncancerous patients, 68 mgm. for the patient with localized cancer, and 125 mgm. for those with metastatic cancer. It is suggested that this finding may result from an accelerated usage of vitamin C by carcinomatous tissue.—Authors' abstract.

TANNENBAUM, A. [Michael Reese Hosp., Chicago, Ill.] THE GENESIS AND GROWTH OF TUMORS. II. EFFECTS OF CALORIC RESTRICTION PER SE. Cancer Research, 2:460-467. 1942.

The effects of a calorie-restricted diet on the genesis and growth of tumors were studied. Observations were made upon spontaneous breast tumor, induced carcinoma, and induced sarcoma of the mouse. Three principal effects were obtained with the calorie-restricted diet: (1) a decrease in the number of tumors formed; (2) a delay in the average time of appearance of tumors; and (3) an increase in longevity of the mice. The restricted diet was restricted in calories only, yet produced the same results as a diet previously used, in which both calories and essential components (protein, fat, vitamins, and minerals) were restricted proportionately. Thus the effects were due to caloric restriction *per se*. Evidence is presented to prove that the formation of spontaneous breast carcinoma in the mouse can be inhibited even if caloric restriction is started just before the tumors begin to appear. The mechanism and significance of these results are discussed.—Author's abstract.

TANNENBAUM, A. [Michael Reese Hosp, Chicago, Ill.] THE GENESIS AND GROWTH OF TUMORS. III. EFFECTS OF A HIGH-FAT DIET. Cancer Research, 2:468-475. 1942.

The effect of a high-fat diet on the genesis of spontaneous breast carcinoma, induced skin tumor, induced sarcoma, and primary lung tumor of the mouse was studied. The fat content of the high-fat diets was from 12 to 31%, in comparison with 2 to 3% for the control diets, which was adequate for normal growth. The most striking result of these investigations was the diversity of effects produced by a high-fat diet: The incidence of spontaneous breast carcinoma was significantly increased; that of the induced skin tumor was increased; the incidence of the primary lung tumor was unaffected; and that of the induced sarcoma was unaffected or actually inhibited. A high-fat diet not only produced a definite increase in the incidence of spontaneous breast and induced skin tumors, but also shortened the mean time of appearance of these tumors. The mean growth rate of sarcomas arising in the high-fat group was not significantly different from that of sarcomas arising in the control group. A 2-fold action of a high-fat diet ("solvent action" and "cocarcinogenic action") is postulated to explain the diverse effects on tumor formation.—Author's abstract.

WARBURG, O., and CHRISTIAN, W. [Kaiser Wilhelm Inst., Berlin-Dahlem, Germany] ISOLIERUNG UND KRISTALLISATION DES GÄRUNGSFERMENTS ENOLASE. [ISOLATION AND CRYSTALLIZATION OF THE RESPIRATORY ENZYME ENOLASE.] Naturwissenschaften, 29:589-590. 1941.

A method is described for the isolation in crystalline form of enolase, an enzyme which promotes the transformation of phosphoglyceric to phosphopyruvic acid. Identification of the enzyme is possible by indirect means, as an increase in the absorption of ultraviolet light at 240 mμ parallels the formation of phosphopyruvic acid. Although the enzyme cannot be crystallized in its free form or its natural combination with magnesium, this does occur in the presence of a mercury salt. The combination with mercury is inactive, but this element can be removed by dialysis against hydrocyanic acid.—M. J. E.

WARBURG, O., and CHRISTIAN, W. [Kaiser Wilhelm Inst., Berlin-Dahlem, Germany] CHEMISCHER MECHANISMUS DER FLUORID-HEMMUNG DER GÄRUNG. [CHEMICAL METHOD OF INHIBITION OF FERMENTATION BY FLUORIDE.] Naturwissenschaften, 29:590. 1941.

Inhibition of the activity of enolase by fluoride results from the formation of a complex magnesium-fluoride-phosphate combination. Depending upon its concentration this substance induces progressive displacement of the activating magnesium salt from its combination with the ferment protein.—M. J. E.

WOODARD, H. Q. [Memorial Hosp., New York, N. Y.] ACID AND ALKALINE GLYCEROPHOSPHATASE IN TISSUE AND SERUM. Cancer Research, 2:497-508. 1942.

A method is described for measuring the action of serum and of crude tissue extracts on sodium-β-glycerophosphate over the pH range from 3.0 to 10.0.

The alkaline glycerophosphatase activity of the cortex of normal adult human long bone has been found to range from 0.04 to 0.15 units per gm. Corresponding values for children's long bone are 0.16 to 3.3 units per gm. Acid phosphatase activities in cortical bone were barely detectible.

Regenerating bone has been found to contain up to 50 times as much alkaline glycerophosphatase as normal bone.

A range of alkaline glycerophosphatase activity from 0 to 115.0 units per gm. has been found in extracts of osteogenic sarcoma tissue. No correlation has been found between phosphatase activity and histological type. Soft-part metastases from osteogenic sarcoma have alkaline glycerophosphatase activities of the same order of magnitude as the primary tumor.

The glycerophosphatases of extracts of giant cell tumors and of endothelioma of bone are active mainly in acid solution. Significant alkaline glycerophosphatase activities are found only when portions of regenerating bone are included in the specimen.

Areas of bone invaded by metastases from tumors of soft part origin contain more alkaline glycerophosphatase than does normal bone.

Acid and alkaline glycerophosphatase activities of the order of 0.5 to 1.5 units per gm. have been found in extracts of normal liver and kidney, and in most specimens of carcinoma and lymphomatoid tissue.

No alkaline glycerophosphatase and very small amounts of acid glycerophosphatase have been found in extracts of normal muscle. Significant amounts of alkaline glycerophosphatase have been found in a few extracts of pathological muscle.

Some evidence has been obtained that the alkaline glycerophosphatase of embryonal adenocarcinoma of the testis is much higher than that of normal testis.

Prostatic glycerophosphatase has been found to be active from pH 2.0 to 8.0. Slight activity was occasionally detected at a pH as high as 8.3, but none in solutions more alkaline than this.

The significance of acid phosphatase readings on the serum of patients with carcinoma of the prostate is discussed.—Author's summary.

Leukemia

KAALAND-JORGENSEN, O. [Municipal Hosp., Aarhus, Denmark] EXPERIMENTS IN TRANSMISSION OF LEUKOSES FROM MICE TO ROENTGEN-IRRADIATED RATS. Acta radiol., 21:483-499. 1940.

This paper records relatively successful attempts to transmit a mouse leukosis of the myeloid type to rats that had received total roentgen irradiation on the day prior to, or on the day of, transplantation. Doses of 450 r had no effect. After the administration of 570 to 625 r the mouse lesion was maintained in one series for 2 generations, and in a 2nd for 11 generations during a period of 89 days.

An additional experiment carried out with leukemia of genetically controlled mouse strains is described, but in this group hetero-transfer was not possible.—M. J. E.

Transplantation

EISEN, M. J. [Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.] THE CONSTANCY UNDER

VARYING CONDITIONS OF A TRANSPLANTED MAMMARY CARCINOMA IN INBRED RATS. Cancer Research, 2: 489-493. 1942.

A tumor which originated in a member of an inbred strain and is maintained in other individuals of the same strain is, because of its uniform behavior, a suitable and efficient medium for observations on the growth of neoplastic cells and the effects of various treatments. The constant and regular growth of a transplanted mammary carcinoma maintained in highly inbred rats is demonstrated in the present paper by the similar proliferative rate of earliest and latest generations of the tumor, and the equal degree of success and almost complete failure of grafts from early and late generations in partially related and totally alien rats respectively. The lungs of partially related and alien rats were not more susceptible than subcutaneous sites.

Grafts of tumors originating from fragments that received 4,000, 4,500 and 5,000 r radiation *in vitro* grew progressively in related rats, but not in alien animals. Under the conditions of the experiment, therefore, radiation did not induce a mutation in the tumor.—Author's abstract.

Cytology

KOLLER, P. C. [Inst. of Animal Genetics, Univ. of Edinburgh, Scotland] A NEW TECHNIQUE FOR MITOSIS IN TUMOURS. Nature, London, 149:193. 1942.

A rapid method is described for the examination of mitosis in tumors. Small pieces of tumor tissue are fixed in acetic alcohol (1:3) for 10 minutes to 24 hours. Thin slices are then cut off and transferred to 10% acetic acid for 5 minutes, followed by 45% for 10 minutes. They are stained with acetic-lacmoid (resorcin-blue), warmed to 40° C. for 15 to 30 minutes. A fragment of stained tissue is placed on a slide in a drop of acetic-lacmoid, and crushed by gently tapping. All unmacerated fragments are next removed, and a cover glass is laid on the remaining tissue and lightly pressed with blotting paper to spread the cells and remove surplus stain. The slide, after being heated, is at once ready for examination. If required for future study slides should be kept for 1 to 5 days in a moist atmosphere at freezing point. For cell-stained permanent preparations it is desirable to leave tissues in acetic-lacmoid for 24 hours.—R. J. L.

Treatment—Radiation, Chemotherapy, etc.

BARDEN, R. P. [Allegheny Gen. Hosp. and Singer Mem. Research Lab., Pittburgh, Pa.] THE INFLUENCE OF ROENTGEN IRRADIATION OF NORMAL LUNG ON THE PREVENTION OF SUBSEQUENT METASTATIC TUMOR GROWTH: PRELIMINARY REPORT. Radiology, 37:608-615. 1941.

A brief review of natural and induced tumor resistance is presented. Experiments are reported on the effect of pulmonary irradiation on the incidence of lung metastases in rabbits inoculated with Brown-Pearce carcinoma. Thirty-four rabbits were inoculated with tumor, usually by the intratesticular route. Five of 6 animals which received no irradiation developed lung metastases. Ten of 19 animals given 300 to 900 r of of roentgen irradiation to the right lung within 1 week before tumor inoculation, developed pulmonary metastases. Only 1 of 7 animals given similar irradiation 2 to 4 weeks before tumor inoculation developed pulmonary metastases. It is concluded that moderate doses of roentgen irradiation decrease the incidence of pulmonary metastases in rabbits subsequently inoculated with Brown-Pearce carcinoma.—C. E. D.

FOGG, L. C., and WARREN, S. [Boston Univ. Sch. of Med., and New England Deaconess and Huntington Memorial Hosps., Boston, Mass.] SOME CYTOLOGIC EFFECTS OF REPEATED DOSES OF RADIATION ON MOUSE SARCOMA 180. Cancer Research, 2:517-520. 1942.

A quantitative study was made of the cumulative effect of repeated doses of 1,200 r at 10-day intervals on the production of abnormal mitoses and multicentriolar cells in mouse sarcoma 180.

Inbred strains of C57 black mice carrying sarcoma 180 were used. After the 3rd radiation the tumor was transplanted into the same strain of mice followed by 2 more radiations at the usual interval. At intervals of 18, 24, 48, 72, 96, and 120 hours after each radiation, tissue was removed for examination. For each preparation a total of 1,000 cells with identifiable centrioles were observed and the number occurring noted. In a like series of 1,000 cells showing mitosis the number of normal cells and those showing some atypical configuration were recorded.

The dosage was sufficient to produce abnormal mitoses in a small percentage of the cells, the highest occurring within 24 to 48 hours after the 1st radiation. After the 3rd radiation there was no appreciable increase in the percentage of abnormal mitoses over that noted in the control.

The 5 doses produced a gradual increase in the percentage of cells revealing more than 2 centrioles. This suggests that one effect of radiation is the prolonged suppression of cleavage without cessation of the function of the kinetic apparatus. Thus while the percentage of abnormal mitoses is more or less constant there is apparently an increase in the number of cells with the capacity to continue through several abortive divisions.—Authors' abstract.

GOLDFEDER, A. [Dept. of Hosps. and New York Univ. Med. Coll., New York, N. Y.] FURTHER STUDIES ON EFFECTS OF IRRADIATION ON PROLIFERATION AND METABOLIC PROCESSES OF NORMAL AND MALIGNANT MAMMALIAN TISSUES. V. EFFECTS PRODUCED BY DIFFERENT DOSAGE RATES OF X-RADIATION UPON GROWTH FACTORS OF MOUSE SARCOMA 180 GROWN IN VIVO FOLLOWING IRRADIATION IN VITRO. Radiology, 37:705-716. 1941.

Mouse sarcoma 180 was irradiated *in vitro* by various technics during the interval between removal from one host and transplantation into a second. It was found that increase in the dose of radiation resulted in a progressive increase in the latent period before tumors became palpable, a decrease in the percentage of takes, and an increase in the percentage of tumor regressions. Tumor tissue irradiated with doses in excess of 2,000 r gave only rare takes with 100% regressions. Greater damage appeared to result from irradiation at a low rate (40 r per minute) than at a higher rate (650 r per minute).

Experiments with 200 kv. and 45 kv. radiation gave similar results for equal doses.—C. E. D.

OVERGAARD, K., and OKKELS, H. [Radium Center, Copenhagen, Denmark] THE ACTION OF DRY HEAT ON WOOD'S SARCOMA. Acta radiol., 21:577-582. 1940.

The tumor employed, a transplantable sarcoma of the mouse, appeared more sensitive to the action of heat administered by diathermy than did the normal tissues. Although exact figures are not given, the authors state that a local temperature of 42-46° C. maintained for 5 to 60 minutes produced some transient and permanent regressions. The administration of combined thermotherapy and roentgen radiation (400 to 2,000 r) was more effective, and resulted in a large number of cures.—M. J. E.

Miscellaneous

CRAMER, W. [Barnard Free Skin and Cancer Hosp., St. Louis, Mo.] THE ORIGIN OF CANCER IN MAN IN THE LIGHT OF EXPERIMENTAL CANCER RESEARCH. Yale J. Biol. & Med., 14:121-138. 1941.

In a general discussion, the practical as well as theoretical importance of knowledge concerning the remote causes of cancer is stressed. Thus exogenous carcinogenic agents may appear as factors in occupational neoplastic disease. Precancerous conditions may serve as a guide in therapy. In such organs as the breast and uterus, where a particular susceptibility to endogenous agents may be familial and where a hormonal imbalance may be causative, there is a further stimulus to clinical observation and to experiment.

The elucidation of the proximate cause; that is, the specific change which transforms a normal into a malignant cell, is another and a still unsolved problem.—A. A. L.

VOEGTLIN, C. [Nat. Inst. of Health, U. S. Public Health Service, Bethesda, Md.] TRENDS IN CANCER RESEARCH. Surg., Gynec. & Obst., 74:561-564. 1942.

A general discussion, not suitable for abstracting.—H. G. W.

Clinical and Pathological Reports

Etiology

COPPS, L. A., and EPSTEIN, S. [Marshfield Clinic, Marshfield, Wis.] TRAUMATIC CANCER OF THE TONGUE. Arch. Otolaryng., 34:1023-1024. 1941.

During a dental maneuver an area of the tongue received a severe injury which did not heal completely. One year later the zone was involved by a squamous cell cancer. As the tongue had been normal prior to the trauma, it is believed that some relationship existed between it and the subsequent neoplastic alteration. The portion of the organ involved was resected.—M. J. E.

RHOADS, C. P. [Memorial Hosp., New York, N. Y.] PRECANCEROUS LESIONS. Surg. Gynec. & Obst., 74:549-551. 1942.

A lecture which emphasizes atrophy as a precursor of cancer, and a report of experiments with butter yellow cancer of the rat liver. The experiments demonstrated that the cancer-producing chemical induced atrophy of liver cells by competing successfully for the protein component of the cells without which their enzyme systems could not operate. Apparently the cancer cell is malignant because it has developed some new way of life which renders it insusceptible to damage by the chemical compound employed in the experiment and concurrently makes it immune to normal growth restraint. Evidence for this hypothesis is presented.—H. G. W.

Heredity

FORMAN, L. FAMILIAL AND CONGENITAL BASAL-CELLED EPITHELIOMA IN THE DISTRIBUTION OF EPITHELIOMA ADENOIDES CYSTICUM, AND SHOWING SOME OF THE HISTOLOGICAL CHANGES OF THE LATTER. Proc. Roy. Soc. Med., 35:261. 1942.

Numerous small nodules appeared at puberty on the chin, nasolabial folds, and in front of the ears in a woman; and in 3 of her 5 daughters and in 1 of her 2 sons. Sections from one of the nodules showed a basal celled tumor, with cells arranged in columns.—E. L. K.

HORNBAKER, J. H. [Washington County Hosp., Hagerstown, Md.] CHRONIC LEUKEMIA IN THREE SISTERS. Am. J. M. Sc., 203:322-325. 1942.

An instance of familial leukemia is reported in which 3 sisters have been affected, 2 with the chronic lymphatic type and 1 with chronic myeloid leukemia.—H. G. W.

Multiple Tumors

TULLIS, J. L. [Bellevue Hosp., New York, N. Y.] MULTIPLE PRIMARY MALIGNANT LESIONS. J. Lab. & Clin. Med., 27:588-594. 1942.

Of 1,044 necropsies on malignant cases, 21 (2%) showed multiple malignant lesions. In 2 patients there were 3 primary malignant lesions. In 12, at least one of the lesions was in the gastrointestinal tract. The amassed data from several large series from American institutions reveal that of 7,488 cases with malignant lesions there were 219 (2.8%) in which the lesions were multiple. This percentage is believed by the author to be higher than can be explained on the basis of chance alone.—H. G. W.

Diagnosis—General

GRAHAM, A. [Cleveland Clinic, Cleveland, Ohio] CRITERIA OF MALIGNANCY. Radiology, 37:521-532. 1941.

The author presents a general discussion of the pathological features characteristic of malignancy and illustrates the difficulties of histological diagnosis with photomicrographs. Treatment and curability of tumors are discussed in relation to histological grade, location, and dissemination.—C. E. D.

THIERSCH, J. B., and SCHLOSSER, S. [Inst. of Med. and Vet. Science, Adelaide, South Australia] THE BROSE-JONES TEST FOR CANCER. M. J. Australia, 1:77. 1941.

The Brose-Jones Test for cancer (Report of the Ninth Australian and New Zealand Cancer Conference, Sidney, April 5th to 9th, 1938), which is based upon the ratio of cell-phosphorus to plasma-phosphorus in the blood, was applied to 1,081 specimens of blood and found to be

"not highly specific and therefore not of great diagnostic value."—E. L. K.

Therapy—General

DAVIDSON, J. R. [Winnipeg, Canada] PRELIMINARY REPORT ON PREVENTION, CONTROL AND TREATMENT OF HUMAN CANCER AS A DEFICIENCY DISEASE. Canad. M. A. J., 45:308-312. 1941.

As may be assumed from the title of this publication, the author treats cancer dietetically. Patients are given either a simple diet rich in vitamins, or, when the lesions are more advanced, this regime is supplemented with additional amounts of vitamins, and injections of an undescribed embryo extract are given to increase resistance. The favorable results obtained in 9 cases are recorded as proof of the value of these methods.—M. J. E.

TURNBULL, F. [Vancouver, Canada] THE PAIN OF CANCER FROM A NEUROSURGEON'S VIEWPOINT. Canad. M. A. J., 45:339-342. 1941.

A discussion of the possible surgical procedures to employ in cases of cancer associated with intractable pain.—M. J. E.

Radiation—Diagnosis and Therapy

ANDREWS, J. R. [Cleveland, Ohio] INTERSTITIAL IRRADIATION IN CANCER OF THE UTERINE CERVIX. Am. J. Roentgenol., 46:700-706. 1941.

The dose distribution of radiation in the pelvis as delivered by various technics of intracavity radium application is considered in detail. It is concluded that these technics as used in the treatment of cancer of the cervix deliver some 4,000 r less than the cancerocidal dose to the lateral pelvic lymph nodes. A technic is described for overcoming this deficiency by the transvaginal implantation of radon seeds.—C. E. D.

BOWING, H. H., and FRICKE, R. E. [Mayo Clinic, Rochester, Minn.] MALIGNANT DISEASE OF THE RECTUM AND RECTOSIGMOID TREATED WITH RADIUM. Radiology, 37:569-574. 1941.

Surgical treatment of carcinoma of the rectum is advised in operable cases but radiation offers palliation and occasional cure in inoperable cases and serves at times as an adjunct to surgery. This article is concerned chiefly with the technic of applying radon seeds, radium needles, and plaques to rectal lesions. The results as previously reported (Am. J. Roentgenol., 32:635-645. 1934) show 39% 3 to 10 year survivals.—C. E. D.

CAHILL, G. F. [Columbia-Presbyterian Med. Center, New York, N. Y.] TUMORS OF THE ADRENAL AND THE USE OF AIR INSUFFLATION IN THEIR DIAGNOSIS. Radiology, 37:533-543. 1941.

A clinical classification of adrenal tumors is proposed, including those of cortical and medullary origin with and without endocrine function.

Adrenal tumors may sometimes be identified in ordinary roentgenograms if the tumor is opaque or if it displaces adjacent organs. Air insufflation of the perirenal fascial planes permits direct visualization of the adrenal contour in most cases. This procedure has been carried out by the author over 400 times without serious mishap and is fairly reliable in revealing the presence and location of adrenal tumors. Air insufflation is contraindicated in large or highly vascular malignant tumors because of the danger of air embolism, hematoma, or dissemination of the tumor. Large tumors can usually be diagnosed by physical examination and pyelography. Reproductions of 22 roentgenograms are presented.—C. E. D.

DOUB, H. P., PRATT, J. P., and JONES, H. C. [Henry Ford Hosp., Detroit, Mich.] COMBINED IRRADIATION AND SURGERY IN THE TREATMENT OF CARCINOMA OF THE PELVIC COLON. Radiology, 37:575-578. 1941.

A method of combined roentgenological and surgical treatment of carcinoma of the pelvic colon is described. Radiation is applied 4 to 6 weeks preoperatively to each of 4 pelvic portals on 4 successive days in doses of 720 r. Irradiation is said to result in improvement of the general condition of the patients, shrinkage of ulcers, occasional increased mobility of the lesion, and a probable decreased danger of postoperative peritonitis. Results on an unstated number of cases treated by this method showed 95% operability and 46% 5 year survivals. Patients treated by surgery alone showed 60% 5 year survivals but this group includes a number of early lesions. Preoperative irradiation appears to increase the operability of cancer of the pelvic colon.—C. E. D.

FRICKE, R. E., and BOWING, H. H. [Mayo Clinic, Rochester, Minn.] FURTHER STUDIES IN THE RADIUM TREATMENT OF CARCINOMA OF THE UTERINE FUNDUS. Am. J. Roentgenol., 46:683-688. 1941.

The treatment of choice in early cases of carcinoma of the cervix is undoubtedly surgical. At the Mayo Clinic 115 cases seen from 1925 to 1935 were considered unsuitable for surgery either because of the extent of the tumor or the presence of concurrent disease. These were treated by intrauterine radium applications with accessory external roentgen treatment in two-thirds of the cases. One hundred and nine cases were traced and showed a 5 year survival of 39%, even including cases in which treatment had to be abandoned or was instituted originally as palliation. The rate of cure with radium treatment varies directly with the extent of the growth and with the microscopic grade of the lesion.—C. E. D.

GOIN, L. S., and HOFFMAN, E. F. [Los Angeles, Calif.] THE USE OF INTRAVESICAL LOW-VOLTAGE CONTACT ROENTGEN IRRADIATION IN CANCER OF THE BLADDER. Radiology, 37:545-548. 1941.

The authors describe a new technic applicable to the treatment of tumors of the trigone of the bladder, less than 3 cm. in diameter. The tumor is exposed by suprapubic cystotomy, biopsied, and fulgurated to the level of the bladder wall. A contact roentgen tube is then introduced through the cystotomy wound and brought into contact with the lesion. A dose of 7,668 r is usually given at 50 kv. and 1,278 r per minute. The treatment is generally repeated once or twice by reopening the bladder. By this technic the field of radiation can be localized accurately to the tumor and the shallow penetration (30% of the surface dose at 1 cm. depth) permits a large surface dose while sparing underlying structures. Eleven of 13 patients treated in this manner showed no evidence of tumor 3 to 19 months after treatment.—C. E. D.

HARE, H. F. [Lahey Clinic, Boston, Mass.] RADIATION TREATMENT OF CARCINOMA OF THE THYROID. Am. J. Roentgenol., 46:451-453. 1941.

The average age of patients with thyroid cancer is 48 years. The ratio of females to males is 5:1. Successful treatment with radiation has been reported over a period of 40 years.

At the Lahey Clinic, radiation treatment is given in all cases following as complete surgical removal of the tumor as is possible. Treatment varies with individual cases but in general a total of 2,000 r is given to each of 3 neck portals over a period of 21 treatment days. Difficulty with wound healing was encountered in only one patient even though treatment was generally begun as soon as possible after surgery. The 5-year survival rates on 231 cases so treated were as follows: fetal adenoma 71%, papillary cystadenoma 62%, papillary adenocarcinoma 80%, alveolar adenocarcinoma 27%, small cell carcinoma 22%, giant cell carcinoma 17%, fibrosarcoma (3 cases) 33%. These results indicate the prognostic importance of the histological diagnosis in thyroid tumors.—C. E. D.

KENNEY, J. M., MARINELLI, L. D., and WOODARD, H. Q. [Memorial Hosp., New York, N. Y.] TRACER STUDIES WITH RADIOACTIVE PHOSPHORUS IN MALIGNANT NEOPLASTIC DISEASE. Radiology, 37:683-687. 1941.

Tracer doses of radioactive phosphorus were administered preoperatively to patients with carcinoma of the breast, osteogenic sarcoma, and lymphosarcoma. Determinations of the radioactivity of tumor tissue removed subsequently revealed a considerable degree of concentration of radioactive phosphorus within the tumors. The results suggest that radioactive phosphorus in therapeutic doses might be effective in the treatment of lymphosarcoma and may be a useful adjunct in treating osteogenic sarcoma and carcinoma of the breast.—C. E. D.

McFARLAND, J. [Univ. of Pennsylvania, Philadelphia, Pa.] THE TREATMENT OF MIXED TUMORS OF THE SALIVARY GLANDS BY ROENTGEN RAYS AND RADIUM. Am. J. Roentgenol., 46:506-517. 1941.

Radiologists generally consider mixed tumors of salivary gland origin as radioresistant yet some surgeons still refer cases for treatment. The author has collected 400 examples of such tumors over a period of 25 years. Sixty-one were treated by radium or roentgen rays and a brief case history of each is given. Radiation treatment failed uniformly. It is considered unjustifiable to subject patients to the expense and discomfort of worthless treatments.—C. E. D.

MANDEVILLE, F. B., RUSSELL, D. A., and FARLEY, M. S. [Med. Coll. of Virginia, Richmond, Va.] ROENTGEN THERAPY OF 100 CONSECUTIVE TUMORS OF THE BRAIN OR SPINAL CORD. Radiology, 37:560-568. 1941.

The authors treated 76 brain tumors, 8 pituitary adenomas, and 16 spinal cord tumors with 200 kv. roentgen radiation in doses of 150 r to over 8,000 r. Most of the cases had been referred as being beyond further neurosurgical benefit. The results are reported in terms of survival, not cure, and are considered separately for medulloblastoma, glioblastoma multiformi, pontine tumors, astrocytoma, ependymoma, craniopharyngioma, pituitary adenoma, unclassified tumors, spongioblastoma polare, hemangioblastoma, hemangioma, meningioma, sarcoma, reticulum cell sarcoma, metastatic tumors, and spinal cord tumors. From the data given, results appear to have been the most promising in medulloblastoma, ependymoma, and spinal cord tumors. Clinical improvement or prolongation of life is claimed for those patients submitting to adequate therapy.—C. E. D.

PENDERGRASS, E. P., and HODES, P. J. [Hosp. of the Univ. of Pennsylvania, Philadelphia, Pa.] FURTHER EXPERIENCES WITH CHAOUL THERAPY. Radiology, 37: 550-559. 1941.

Short distance, low voltage roentgen rays may be delivered at high intensity but give a small depth dose. They are thus suited to the treatment of superficial neoplastic and inflammatory lesions. The authors discuss the physical factors involved in the production and absorption of these rays and cite the indications and contraindications for their clinical use. In general, treatment should be limited to superficial lesions of small or moderate size so situated that cartilage need not be included in the field. Dosage varies from 2,000 to 6,000 r delivered in one or several sittings depending upon the reaction of the area treated. The authors have treated 541 lesions including 228 malignant tumors by this method with satisfactory results. They consider it an efficient and economical means of delivering large quantities of radiation to small volumes of superficial tissue.—C. E. D.

PORTMANN, U. V. [Cleveland Clinic, Cleveland, Ohio] EXPERIENCES IN THE TREATMENT OF MALIGNANT TUMORS OF THE THYROID GLAND. Am. J. Roentgenol., 46:454-466. 1941.

The five year results of the treatment of 220 cases of malignant thyroid tumors are presented. Of the patients, 70% were in an advanced stage of disease when first seen. At least 80% of the tumors originated in preexisting adenomas. Tables are given classifying the tumors according to histological type and extent of involvement and showing the results of therapy by surgical and radiological measures. Surgical treatment alone was adequate in tumors confined within the capsule of the gland and postoperative irradiation conferred no added benefit. Irradiation probably prolonged the lives of some patients with extensive involvement.—C. E. D.

RYPINS, E. L. [Bloomington, Ill.] THE ROENTGENOLOGIC ASPECTS OF SUBUNGUAL GLOMUS TUMOR. Am. J. Roentgenol., 46:667-672. 1941.

The author discusses the anatomy of the normal cutaneous glomus and the tumors arising from this structure. A case of subungual glomus tumor is presented together with 4 roentgenograms showing bone defects in the terminal phalanx of the affected digit. Such roentgenograms associated with a history of severe pain are considered diagnostic of glomus tumor.—C. E. D.

SCHATZKI, R. [Massachusetts Gen. Hosp., Boston, Mass.] ROENTGENOLOGICAL DIAGNOSIS OF PRIMARY CARCINOMA OF THE LIVER. Am. J. Roentgenol., 46:476-483. 1941.

Fifteen primary carcinomas of the liver were found among 4,323 autopsies at the Massachusetts General Hospital. Eleven were liver cell carcinoma and 4 were cholangiomas. Ten of the liver cell carcinomas and 3 of the cholangiomas were associated with cirrhosis. A case is reported in which primary carcinoma of the liver was diagnosed roentgenologically. The roentgenological

findings in 3 other cases not diagnosed during life are discussed. The roentgenologist may suspect primary carcinoma of the liver when he can demonstrate cirrhosis of the liver (esophageal varices, large spleen with small liver) associated with an abnormal mass in the region of the organ. The demonstration of metastases fortifies the diagnosis.—C. E. D.

SIMS, G. P. [Mt. Carmel Hosp., Columbus, Ohio] HIGH-VOLTAGE ROENTGEN IRRADIATION OF ACCESSIBLE CANCER OF SKIN AND MUCOUS MEMBRANE. Radiology, 37:666-672. 1941.

Roentgen rays generated at 200 kv. or more deliver a more homogeneous and effective depth dosage than softer rays and are preferred by the author for the treatment of superficial cancer. Six cases are presented illustrative of the satisfactory results obtainable with high voltage therapy.—C. E. D.

ZAHL, P. A., and COOPER, F. S. [Memorial Hosp. and Haskins Labs., New York, N. Y.] PHYSICAL AND BIOLOGICAL CONSIDERATIONS IN THE USE OF SLOW NEUTRONS FOR CANCER THERAPY. Radiology, 37:673-682. 1941.

Slow neutrons in traversing ordinary tissues produce little destructive ionization. However, lithium or boron atoms placed in the path of such a beam result in neutron capture and local release of ionizing energy. The localization of lithium or boron within a tumor would thus result in selective damage to the tumor on exposure to a beam of slow neutrons.

The authors show that certain lithium-containing dyes, injected intravenously, are selectively concentrated in the peripheral portions of experimental mouse tumors. Concentrations of metallic ions were obtained theoretically sufficient to give a 2:1 ratio of energy release in the tumor as compared to normal tissue. The difference in biological effect may be even greater than indicated by the 2:1 ratio, caused by greater density of ionization. Further work is in progress.—C. E. D.

Skin and Subcutaneous Tissues

ACKERMAN, L. V., and WHEELER, P. [Ellis Fischel State Cancer Hosp., Columbia, and St. Louis City Hosp., St. Louis, Mo.] LIPOSARCOMA. South. M. J., 35:156-159. 1942.

Report of 3 typical cases, 2 arising in the interfascial planes of the lower limbs and the third in the pleural cavity.—H. G. W.

HANSSON, C. J. [Radiumhemmet, Stockholm, Sweden] KAPOSI'S SARCOMA: CLINICAL AND RADIOTHERAPEUTIC STUDIES ON TWENTY-THREE PATIENTS. Acta radiol., 21: 457-470. 1940.

Kaposi's sarcoma of the skin, in the 23 cases described by the author, proved extremely radiosensitive. Radium was applied to smaller lesions, roentgen radiation being reserved for the more extensive or infiltrative types. Although the disease regressed in all cases, 7 patients subsequently died of intercurrent affections.—M. J. E.

PACK, G. T., and WUESTER, W. O. [Memorial Hosp., New York, N. Y.] THE DEVELOPMENT OF CANCER IN ACRODERMATITIS CHRONICA ATROPHICANS. J. A. M. A., 118:879-884. 1942.

Report of 4 instances of this rare condition, found among 3,000 cases of epitheliomas of the skin.—H. G. W.

TEMPLETON, H. J. [Oakland, Calif.] CUTANEOUS AND MUCOUS MEMBRANE CANCER. California & West. Med., 55:81-83. 1941.

An analysis of the results of treatment of cancer of the skin and mucous membrane is reported. Since the author is a confirmed believer in the superiority of electrosurgery—either actual cautery or excision—over radiotherapy, destruction of the tumors by the former technic was employed in 71% of the cases. Of 1,131 patients with cancer of the skin 98.7% were cured. Among 92 patients with cancer of the lower lip without palpable metastases, of the 89 who were cured, 46 had been treated by electrosurgical methods alone, while the others had received supplementary radiotherapy. The cosmetic results in the irradiated patients appeared inferior. All 31 patients with cancer of the upper lip treated by either method or a combination of both were classified as tumor-free. Electrocoagulation, generally supplemented by radium implantation or roentgen therapy, was employed in 29 cases of cancer of the tongue or oral mucosa without metastatic foci; 19 patients in this group were known to be cured. Six patients with metastases at the time of therapy died. Recurrences appeared likewise to be influenced favorably by electrodesiccation. In all groups the interval following the conclusion of therapy varied from less than 2 to more than 5 years.—M. J. E.

Nervous System

COATES, G. M. [Philadelphia, Pa.] SCHWANNOMA OF THE MOUTH. Arch. Otolaryng., 34:1166-1167. 1941.

A case report.—M. J. E.

HALL, C., and OWENS, H. [Univ. of Southern California Sch. of Med., Los Angeles, Calif.] SOLITARY NEUROFIBROMA OF THE PHARYNX. Arch. Otolaryng., 34:1163-1165. 1941.

The tumor was excised from the peritonsillar area.—M. J. E.

HALPERN, L. [Rothschild Hadassah Univ. Hosp., Jerusalem, Palestine] A REMARKABLE CASE OF SPINAL METASTASIS IN A CEREBELLAR MEDULLOBLASTOMA. J. A. M. A., 118:803. 1942.

A boy of 7 was operated upon for a midline cerebellar medulloblastoma. X-rays 15 months later revealed 2 Cushing silver clips used in the operation, displaced in the spinal canal at the 5th lumbar level; and operation 3 months later disclosed the spinal canal at this level to be filled with tumor tissue.—H. G. W.

POTTER, E. L., and PARRISH, J. M. [Univ. of Chicago and Chicago Lying-in Hosp., Chicago, Ill.] NEUROBLASTOMA, GANGLIONEUROMA AND FIBRONEUROMA IN A STILLBORN FETUS. Am. J. Path., 18:141-151. 1942.

This is a case report of a stillborn premature fetus in which there was generalized neoplasia of the sympathetic ganglia, the adrenal glands, and the urinary bladder. Tumor foci were present in the liver. The sympathetic and spinal nerves were hypertrophied. The vagus nerve and the sympathetic ganglia contained cells in all stages of differentiation from sympathogonia to mature ganglion cells. In addition there were irregular bundles of fine fibers presumably derived from the sheath of Schwann. The adrenal glands were the site of a tumor which bridged across the midline connecting the 2 organs; the structure of the tumor tissue was similar to that described

above. A neurofibroma extended from the umbilicus to the wall of the bladder and was confluent from there to the sigmoid colon and rectum, terminating in the hypogastric plexus. Multiple foci of sympathicoblasts were present within the liver; these were considered to have arisen from the chromaffin cells normally found there.

The "close interrelationship of ganglioneuromas, neuroblastomas and fibroneuromas and the common identity of the stimulus required for their production" is illustrated in this case.—H. B.

SCHWARTZ, A. A., and ISAACS, H. J. [New York, N. Y.] GLIOMA (ASTROCYTOMA) OF THE NARES. Arch. Otolaryng., 34:838-843. 1941.

An astrocytoma, completely blocking the nasal orifice of an infant of 13 months, had been present since birth. It was extirpated and had not recurred 10½ months later.—M. J. E.

WYATT, G. M., and FARBER, S. [Children's Hosp., and Harvard Med. Sch., Boston, Mass.] NEUROBLASTOMA SYMPATHETICUM. ROENTGENOLOGICAL APPEARANCES AND RADIATION TREATMENT. Am. J. Roentgenol., 46: 485-495. 1941.

Forty neuroblastomas were found among 301 malignant tumors histologically verified at the Boston Children's Hospital during the 10 year period ending in 1939. A previous report (Farber, Neuroblastoma, abstract in Am. J. Dis. Child., 60:749-751. 1940) discusses the life history, treatment, and prognosis of these cases. Thirty-four of the patients were studied roentgenologically. Primary tumors were most frequently found in the posterior abdomen but not always in relation to the adrenal medulla. The posterior mediastinum was the next most frequent site. The remainder arose from the sympathetic chain anywhere from the neck to the pelvis. No basis was found for dividing these tumors into Pepper and Hutchinson types. Metastases were widespread, often bilaterally symmetrical, and were found principally in bone. Combined bone destruction and proliferation was present in the majority of lesions, sometimes simulating leukemic infiltration or osteogenic sarcoma. Of the 40 cases, 10 have survived 3 to 8 years and 5 of these received radiation therapy. Radiation therapy, beginning with small doses, is advised in all cases. The prognosis is not hopeless even in the presence of metastases.—C. E. D.

Eye

CARLBERG, O. [Upsala, Sweden] MELANOSIS BULBI MIT MELANOSARKOM. [MELANOSARCOMA COMPLICATING MELANOSIS BULBI.] Acta ophth., 18:301-308. 1940.

Two cases are described, in which ocular melanoma appeared secondary to melanotic deposits of many years' duration. The first patient had a malignant tumor of the choroid complicating diffuse melanosis of the sclera, the second a neoplasm of an iris which always had been darkly pigmented. Enucleation was performed in both cases.—M. J. E.

GODTFREDSEN, E. [Radiumstation, Copenhagen, Denmark] AUGENSYMPTOME BEI MALIGNEN RHINOPHARYNX-TUMOREN. [EYE SYMPTOMS SECONDARY TO MALIGNANT TUMORS OF THE NASOPHARYNX.] Acta ophth., 18:336-354. 1940.

Ocular manifestations, usually the result of infiltration of the base of the skull, were observed in 20 of 64 patients with cancer of the nasopharynx. Palsy resulting from the involvement of the abducens nerve occurred most commonly, while involvement of the oculomotor, trochlear, and optic nerves was less frequent. Exophthalmus appeared rarely. Horner's syndrome developed occasionally in patients with metastases to the cervical lymph nodes.—M. J. E.

Ear

PEELE, J. C., and HAUSER, G. H. [Eye, Ear, Nose and Throat Hosp., New Orleans, La.] PRIMARY CARCINOMA OF THE EXTERNAL AUDITORY CANAL AND MIDDLE EAR. REVIEW OF THE LITERATURE; REPORT OF A CASE OF CYSTIC ADENOID EPITHELIOMA (BROOKE'S TUMOR) OF THE EXTERNAL AUDITORY CANAL. Arch. Otolaryng., 34:254-266. 1941.

Following a local excision of a Brooke's tumor of the external auditory canal, it was necessary to perform a radical extirpation for a recurrence.—M. J. E.

Breast

IRVINE, A. D. [Edmonton, Canada] AN ANALYSIS OF PUBLISHED STATISTICS IN CONNECTION WITH MAMMARY CANCER. Canad. M. A. J., 45:42-46. 1941.

Analysis of his own cases and those of other investigators leads the author to the conclusion that, while radiotherapy does little to improve the results of surgical treatment of early mammary cancer, combined methods of treatment or radiotherapy alone elicit a better response from the patients with more advanced tumors.—M. J. E.

SCHENCK, S. G. [Brooklyn, N. Y.] CLASSIFICATION OF CANCER OF THE BREAST. Am. J. Roentgenol., 46: 709-718. 1941.

A new clinical classification of carcinoma of the breast is proposed. It covers the same major features as other classifications but in greater detail.—C. E. D.

SIMMONS, C. C. [Boston, Mass.] CANCER OF THE BREAST. TEN YEAR END-RESULTS. Surg., Gynec. & Obst., 74:763-765. 1942.

A report on the 10 year end-results of radical operation for cancer of the breast at the Massachusetts General Hospital during the 5 year period, 1927 to 1931 inclusive, the same cases having been previously reported on a 5 year basis. It would appear from this analysis that in cases of cancer of the breast in which the axillary nodes are not diseased, patients living without clinical evidence of cancer at the end of 5 years may be considered as permanent cures, but in patients with positive nodes at the time of the operation, 19% living at the end of 5 years will eventually die of late recurrence.—H. G. W.

Urinary System—Male and Female

HAMM, F. C. [Brooklyn Hosp., Brooklyn, N. Y.] WILMS' TUMOR IN A 64 YEAR OLD MALE: REPORT OF A CASE. J. Urol., 47:403-409. 1942.

Report of the 26th recorded case in an adult of this tumor, which is ordinarily seen only in childhood.—H. G. W.

JOHNSON, C. M., and SMITH, D. R. [Univ. of California Med. Sch., San Francisco, Calif.] BENIGN POLYPS OF THE URETER. J. Urol., 47:448-452. 1942.

A case is added to the 53 found in the literature.—H. G. W.

NICHOL, J. E. [Lockwood Clinic, Toronto, Canada] TWO CASES OF CARCINOMA OF THE URETHRA. Canad. M. A. J., 45:155-156. 1941.

In the first patient, a female with an adenocarcinoma, the tumor was excised, while in the second, a male with a squamous cell cancer, it was necessary to resort to amputation of the penis. Both patients received radiotherapy after operation.—M. J. E.

WEISEL, W., and DOCKERTY, M. B. [Mayo Clinic, Rochester, Minn.] ADENOCARCINOMA AND FIBROSARCOMA IN THE SAME KIDNEY: REPORT OF TWO CASES. J. Urol., 47:410-415. 1942.

Report of 2 cases of this rare combination, both in adult males.—H. G. W.

Oral Cavity and Upper Respiratory Tract

FRANK, D. I. [New York, N. Y.] LEIOMYOSARCOMA OF THE LARYNX. REPORT OF A CASE. Arch. Otolaryng., 34:493-500. 1941.

The tumor was removed in 2 stages through a laryngoscope, and the patient was given intense roentgen therapy to each side of the neck. There was no recurrence after 18 months.—M. J. E.

HAVENS, F. Z., and PARKHILL, E. M. [Mayo Clinic, Rochester, Minn.] TUMORS OF THE LARYNX OTHER THAN SQUAMOUS CELL EPITHELIOMA. Arch. Otolaryng., 34:1113-1122. 1941.

Approximately 1 in 45 malignant laryngeal tumors is not a squamous cell cancer. This report is based on an analysis of 26 of the more unusual types of neoplasms, of which 11 were sarcomas, 8 hemangioendotheliomas, 5 adenocarcinomas, and 1 each a melanoma and a plasma cell myeloma. Although there may be special clinical features characteristic of each type, diagnosis generally awaits histologic examination of the tissues. Sarcoma is likely to be pedunculated and noninfiltrative. Surgery or radium is the effective method of treatment; and 8 of 11 patients with sarcoma were cured, 1 died of a recurrence, and 2 intercurrently without evidence of tumor. Hemangioendothelioma is likewise relatively benign. Hoarseness is the outstanding symptom, and the localized growth may be excised easily with the aid of the laryngoscope. Endoscopic removal was performed in 7 patients, of whom 5 were cured, 1 died of a recurrence, and one of an independent gastric cancer. A similar tumor was discovered postmortem in an infant of 2 months with a history of increasing dyspnea. Adenocarcinoma is more malignant and demands radical surgical measures. Of 5 patients with this tumor, 2 had inoperable growths when examined, 2 developed metastases after operation, and 1 appeared cured. Melanoma also is malignant, and extensive metastases were present in the patient with this tumor. In the 1 case of myeloma the lesion was confined to the larynx, and the patient remained symptom-free 4½ years after laryngoscopic excision of a mass from the aryepiglottic fold. Photomicrographs are reproduced.—M. J. E.

HUGHES, T. E. [Richmond, Va.] NASOPHARYNGEAL FIBROMA. REPORT OF A CASE. Arch. Otolaryng., 34: 57-68. 1941.

In the case reported, a large tumor was removed surgically.—M. J. E.

IMPERATORI, C. J. [New York, N. Y.] LYMPHOMA OF THE LARYNGOPHARYNX. Arch. Otolaryng., 34:1168-1170. 1941.

The tumor, histologically a lymphosarcoma, was attached to the aryepiglottic fold and was readily excised.—M. J. E.

MOTLEY, F. E. [Charlotte Eye, Ear and Throat Hosp., Charlotte, N. C.] POSITION MALIGNANCY. Arch. Otolaryng., 34:771-786. 1941.

Three of the 5 lesions discussed were neoplastic. While histologically benign or questionably malignant, they possessed the clinical features of malignancy, either because of their location in vital areas, or because of the technical difficulties encountered in removal. In the latter category were a neurofibroma of the pharynx and a myxochondroma of the nasofrontal region. Of the former type was an adamantinoma of the jaw which recurred repeatedly after operation and eventually proved fatal as a result of intracranial extension. Photographs and photomicrographs are reproduced.—M. J. E.

Salivary Glands

SINGLETON, A. O. [Univ. of Texas, Galveston, Tex.] TUMORS OF THE SALIVARY GLANDS, BENIGN AND LOCALLY MALIGNANT. Surg., Gynec. & Obst., 74:569-572. 1942.

Of 58 tumors arising primarily in the salivary glands, 43, or 74%, were mixed tumors, and 15, or 26%, were carcinoma. The age of onset averaged for patients with mixed tumors 31.5 years, and for those with the malignant tumors 52.3 years. Of the mixed tumors operated on in the John Sealy Hospital, only 8.7% recurred, which indicates the need for conservatism in operating on these tumors when they are so located as to cause facial paralysis by operative injury to the facial nerve.—H. G. W.

Intrathoracic Tumors—Lungs—Pleura

FOSTER-CARTER, A. F. [Brompton Hosp., London] BRONCHIAL ADENOMA. Quart. J. Med., 10:139-174. 1941.

Bronchial adenomas form a distinct clinical group of tumors accounting for about 5% of all bronchial neoplasms detected by bronchoscopy. They are the commonest benign tumors of the bronchus. Twenty-two examples are described as occurring in a series of 453 proved cases of bronchial neoplasm. Symptomatology, physical findings, pathology, prognosis, and treatment are fully discussed, and the cases are compared with those described in the literature.

Evidence is produced to suggest that bronchial adenomas are probably identical in nature with salivary gland tumors. They all show certain histological characteristics, the chief being uniformity of structure and staining properties, a tendency to glandular formation—although only about a third of all bronchial adenomas have the highly differentiated glandular structure which has previously been regarded as typical of these tumors—and absence of unruly growth. Limited infiltration of the bronchial wall has been observed, but metastatic spread is unknown and extension of the tumors is never the cause of death.—A. H.

HOLLEB, H. B., and ANGRIST, A. [Queens Gen. Hosp., Jamaica, N. Y.] BRONCHIOGENIC CARCINOMA IN ASSOCIATION WITH PULMONARY ASBESTOSIS. Am. J. Path., 18:123-135. 1942.

Eight cases of bronchiogenic carcinoma in association with pulmonary asbestosis have been reported in the literature. The authors describe two additional ones. Data from the ten cases reveal that the patients had had exposures to asbestos dust for from 7 to 25 years; they ranged from 35 to 71 years of age; 7 of them were males. Histologically 7 of the tumors were squamous cell, 2 were oat cell, and 1 was glandular in type.—H. B.

JONES, T. E., VAN ORDSTRAND, H. S., and PAXTON, J. R. [Cleveland, Ohio] SURGICAL TREATMENT OF PRIMARY CARCINOMA OF THE LUNG. Surg. Clin. North America, 21:1405-1429. 1941.

Findings at necropsy have revealed the lungs to be a far more common site of primary malignant growth than has been appreciated previously. Approximately 10% of all primary cancers originate in the lungs. Between 75 and 90% of all primary carcinomas of the lungs are bronchogenic, the remainder being of peripheral origin. The symptomatology and diagnosis of primary carcinoma of the lung is discussed. Under diagnosis are considered: physical signs, bronchoscopic examination, aspiration biopsy, and exploratory thoracotomy. Irradiation and surgical intervention are discussed under treatment. Eight case histories are given in detail.—J. L. M.

KING, A. B., and FORD, F. R. [Johns Hopkins Hosp., Baltimore, Md.] A CLINICAL AND ANATOMICAL STUDY OF NEUROLOGICAL CONDITIONS RESULTING FROM METASTASES IN THE CENTRAL NERVOUS SYSTEM DUE TO CARCINOMA OF THE LUNG. REVIEW OF ONE HUNDRED CASES. Bull. Johns Hopkins Hosp., 70:124-156. 1942.

A study of 100 cases of carcinoma of the lung in which the nervous system has been examined postmortem revealed 27 with nervous system metastases. The metastases were multiple in 20, and usually less than 1 cm. in diameter. There were 8 metastases of more than 2 cm. In 4 instances deposits were found in the hypothalamus and in 2, the anterior lobe of the hypophysis was involved. The adrenals were involved in more than half the cases, whereas the thyroid was involved but twice. In certain cases the primary growth was so small that it could not be detected by clinical means. In this series the neurological features were predominant in 14.

A roentgenological examination of the chest should be made in all instances in which there is reason to suspect a cerebral or spinal cord neoplasm or in which there is unexplained stupor.—H. G. W.

MOORE, P. M. [Cleveland, Ohio] THE ROLE OF BRONCHOSCOPY IN CARCINOMA OF THE LUNG. Surg. Clin. North America, 21:1431-1441. 1941.

The chronologic symptomatology of carcinoma of the lung is definite and knowledge of it is valuable in an early diagnosis. The role of bronchoscopy in making the earliest possible diagnosis, and in supplying the thoracic surgeon with information relative to operability is discussed.

Two cases are presented in which the infection in the lung secondary to obstruction of a bronchus by the carcinoma had led to a clinical diagnosis of abscess and tuberculosis until bronchoscopy revealed the presence of a growth in the bronchus, and biopsy done through the bronchoscope had established the diagnosis of carcinoma of the lung.—J. L. M.

WHITE, T. J., COHEN, S., GNASSI, A. M., and PRICE, P. [Med. Center, Jersey City, N. J.] PRIMARY CARCINOMA OF THE BRONCHUS. AN ANALYSIS OF FIFTY-SIX CASES. J. A. M. A., 118:862-865. 1942.

An analysis of 56 histologically proved cases, 37 of which were confirmed by necropsy, with a correct antemortem diagnosis in 80%. Men were 88% of the patients. Surgical intervention was unsuccessful in every instance, and radiation therapy was of no benefit. No significant correlation was noted between the histological features and the clinical course.—H. G. W.

WHITESIDE, W. C. [Edmonton, Canada] PRIMARY CARCINOMA OF THE LUNG—PNEUMONECTOMY. Canad. M. A. J., 45:436-438. 1941.

A patient was free from symptoms for 18 months following pneumonectomy for cancer of the bronchus.—M. J. E.

Heart

ORR, J. W. [University of Leeds, England] ENDOTHELIOMA (PSEUDOMYXOMA) OF THE HEART. J. Path. & Bact., 54:125-128. 1942.

A polypoid growth of the left auricle of the heart is described. The tumor almost completely filled the auricle, and was attached by a thin stalk to the region of the scar of the foramen ovale. The author points out that it is many years since the association of endothelial and myxoid elements in cardiac tumors was first recognized, although later work has tended to lay stress on the latter component. He regards it as probable that some of these tumors at any rate are essentially endotheliomatous, the "myxomatous" process being a secondary change of non-neoplastic nature. That the myxomatous tissue is not very convincingly neoplastic in appearance is shown by the frequency with which various authors have suggested the alternative diagnosis of organized thrombus.—A. H.

Liver

BERMAN, C. [Univ. of Witwatersrand, Johannesburg, South Africa] THE ETIOLOGY OF PRIMARY CARCINOMA OF THE LIVER—WITH SPECIAL REFERENCE TO THE BANTU RACES OF SOUTH AFRICA. S. African J. M. Sc., 6:145-156. 1941.

The last of a series of 4 papers on the geographical distribution of primary cancer of the liver, and on the pathology, clinical features, and etiology of this disease as seen in the Bantu of South Africa (Cancer Research, 1:176-177, 915. 1941), with a valuable bibliography of 123 references. The possible etiological factors considered are:

1. *Cirrhosis.*—In young Bantu mine laborers in Johannesburg this was found in 10% of East Coast natives, and in 2.5% of South African natives; in the former, primary cancer of the liver is 6 times more frequent than in the latter. Cirrhosis was present in every one of 25 cases of primary liver cancer examined by the author, and in 18 out of 29 cases examined at another hospital. Cirrhosis is less frequent in Europe and America than in

some countries (East Africa, Java) where cancer of the liver is common. Cirrhosis was present in 405 of 555 cases of primary liver carcinoma recorded in the literature.

2. *Parasitic infestation.*—Helminthiasis is frequent among the natives in question. The literature relating to an association between schistosomiasis and liver cancer is reviewed. The ova of *Schistosoma haematobium* were found at autopsy in the bladder in 24 out of 54 cases of primary liver cancer studied by the author; no ova were found in 25 cancerous livers. Distomiasis has not been observed in the Bantu, and no cases of liver cancer in association with hydatid cysts have been recorded in South Africa. Schistosomiasis is very prevalent in Egypt, where primary liver cancer is rare. In Brazil, Davis examined 29,593 human livers, and found in 1,594 of them lesions caused by *S. mansoni* (cirrhosis and pigmentation), but there were no cases of primary cancer. Helminthiasis cannot account for the prevalence of liver cancer in Java and Sumatra (Bonne).

3. *Syphilis.*—The Wassermann test was positive in only 8 out of 36 cases.

4. *Hemochromatosis.*—The liver in every case of liver cancer examined by the author gave a strong hemosiderin reaction, especially in the cirrhotic areas. In South Africa hemochromatosis occurs more frequently than in Europe, and oftener in Bantu than in whites; in 3 out of 33 cases in Bantu, liver cancer was present also. Hemochromatosis is always accompanied by cirrhosis.

5. *Alcohol.*—". . . . all African native races are accustomed to the consumption of alcoholic beverages from early childhood." Fermented preparations of millet (Kaffir Beer) and maize (Marewu) are used. "Kaffir Beer and Marewu are taken all the year round by Bantu men, women, and children who regard them both as a food and a drink." This early exposure to the effects of alcohol is of interest in relation to the early age at which liver cancer appears. "In the Bantu 82.6 per cent of all cases were forty years of age and under, the highest figure (44.4 per cent) being recorded in the decennium twenty-one to thirty years."

6. The Bantu show a "keloid diathesis" in that considerable connective tissue hyperplasia follows slight superficial wounds; possibly the liver is in some similar way specially reactive to injurious factors (on the proportion of binucleated cells in the Bantu liver see Gillman, South African J. M. Sc., **5**:46. 1940).

The data available indicate that the prevalence of liver cancer in the Bantu depends upon a combination of racial and environmental factors.—E. L. K.

LISA, J. R., SOLOMON, C., GORDON, E. J. [City Hosp., Welfare Island, Dept. of Hosps., New York, N. Y.] SECONDARY CARCINOMA IN CIRRHOSIS OF THE LIVER. Am. J. Path., 18:137-140. 1942.

Only 5 authentic cases of malignant disease metastasizing to cirrhotic livers were found in the literature by the authors. They report 6 additional ones with primary sites as follows: stomach 2, pancreas 2, papilla of Vater 1, esophagus 1. The route of spread to the liver was hematogenous, lymphogenous, or by direct contiguity. They state that the rarity of metastases in cirrhosis may be due either to the infrequent association of cirrhosis with extrahepatic malignancy or because the cirrhotic liver is poor soil for metastatic tumor cells.—H. B.

Bone and Bone Marrow

CARMICHAEL, F. A., HELWIG, F. C., and WHEELER, J. H. [St. Lukes Hosp., Kansas City, Mo.] CRANIAL CHORDOMA. Am. J. Surg., 55:583-587. 1942.

Report of a case in a woman of 47 who was strikingly benefited, at least temporarily, by surgical intervention.—H. G. W.

GILMORE, G. B. [Morrisania City Hosp., New York, N. Y.] MULTIPLE MYELOMA WITH LARYNGEAL METASTASIS. Arch. Otolaryng., 34:453-460. 1941.

Since invasion of the larynx by plasma cell myeloma is unusual, it was assumed that a patient with evidence of a laryngeal tumor had a primary cancer. The correct diagnosis was revealed by a histologic examination of the excised mass, and was further substantiated by the presence of neoplastic cells in the sternal marrow and roentgen evidence of involvement of the skull.—M. J. E.

INCLAN, A. [Havana Univ., Havana, Cuba] THE POSSIBILITIES OF THE ROENTGENOGRAPHIC STUDY OF THE ARTERIAL CIRCULATION IN THE EARLY DIAGNOSIS OF BONE MALIGNANCY. J. Bone & Joint Surg., 24:259-269. 1942.

Arteriography is of importance and practical value in the differential diagnosis of borderline lesions of bone. It will reveal and clearly determine the malignant or benign character of the lesion.—H. G. W.

SHARPE, W. S., and McDONALD, J. R. [Mayo Clinic, Rochester, Minn.] REACTION OF BONE TO METASTASIS FROM CARCINOMA OF THE BREAST AND THE PROSTATE. Arch. Path., 33:312-325. 1942.

Of 219 metastases from breast cancer, 97.3% were osteoclastic, whereas of 66 metastases of cancer of the prostate 97% were osteoplastic, but it was always possible to demonstrate both processes in the same lesion. In the osseous metastases from carcinoma, almost invariably large amounts of fibrous tissue were observed which could be demonstrated to be undergoing transformation into osteoid tissue. Fibrous tissue was seen in smaller quantities in the osseous lesions secondary to cancer of the breast. Cancer of the breast of higher grades of malignancy showed more tendency to metastasize to bone than that of lower grade, but a similar difference could not be detected in prostatic cancer.—H. G. W.

WISE, I. M. [Mobile, Ala.] OSTEITIS FIBROSA CYSTICA. South. Surgeon, 10:819-824. 1941.

A case report of regression of osteitis fibrosa cystica following extirpation of an adenoma of the parathyroid gland.—M. J. E.

Blood Vessels

BAILEY, O. T., and FORD, R. [Harvard Med. Sch., Peter Bent Brigham and Boston City Hosps., Boston, Mass.] SCLEROSING HEMANGIOMAS OF THE CENTRAL NERVOUS SYSTEM. PROGRESSIVE TISSUE CHANGES IN HEMANGIOBLASTOMAS OF THE BRAIN AND IN SO-CALLED ANGIOBLASTIC MENINGIOMAS. Am. J. Path., 18:1-27. 1942.

There have been described 2 groups of vascular neoplasms of the central nervous system: the hemangioblastomas of the brain substance, which occur most com-

monly in the cerebellum, and the angioblastic meningiomas, which are hemangiomas of the meninges. The authors show that these are fundamentally the same tumor; namely, a hemangioma in which sclerosis has occurred in much the same way that Wolbach (1913) observed in cutaneous hemangiomas giving rise to "giant celled xanthomas" and "histiocytomas." In those of the central nervous system the stromal elements that are increased in amount consist both of collagenous connective tissue and neuroglia; the latter arise after there has been invasion of the brain substance by the tumor. The vessels are occluded, and hemosiderin and lipid material accumulate within phagocytic cells of endothelial origin producing yellow and brown zones within and around the vascular tumor. These sclerosing hemangiomas can be recognized during surgical procedures by these gross characteristics; removal of some of the adjacent apparently normal brain tissue is then indicated because of the invasive tendency of this type of tumor.—H. B.

BALLANTYNE, A. J. [Tennent Inst. of Ophthalmology, Univ. and Western Infirmary, Glasgow, Scotland] ANGIOMATOSIS RETINAE. ACCOUNT OF A CASE, INCLUDING THE HISTOLOGICAL RESULTS OF X-RAY TREATMENT. Proc. Roy. Soc. Med., 35:345-357. 1942.

An account of a vascular tumor of congenital origin occurring near the macula in a man of 19. Fundus drawings show the appearances before and after x-ray treatment, and the histology of the retina and its vessels is shown in photomicrographs of sections obtained after excision of the eye following intraocular hemorrhage and secondary glaucoma.—A.H.

KLEINBERG, S. [New York, N. Y.] ANGIOMA OF THE FOOT. J. Bone & Joint Surg., 24:367-371. 1942.

Report of a case of benign capillary hemangioma of the cuboid and external cuneiform bones and soft tissue in the sole of the foot, in a girl aged 19.—H. G. W.

Leukemia, Lymphosarcoma, Hodgkin's Disease

GORHAM, A. T., ABELS, J. C., ROBBINS, A. L., and RHOADS, C. P. [Memorial Hosp., New York, N. Y.] THE MEASUREMENT AND METABOLISM OF THIAMIN AND OF A PYRIMIDINE STIMULATING YEAST FERMENTATION FOUND IN THE BLOOD CELLS AND URINE OF NORMAL INDIVIDUALS. J. Clin. Investigation, 21:161-176. 1942.

Methods for the quantitative determination of thiamin and the pyrimidine accelerator of yeast fermentation have been adapted for application to the white cells and erythrocytes of normal individuals. The technics used depend upon the principle that within certain limits both thiamin and the pyrimidine accelerator cause a measurable increase in the rate of alcoholic fermentation by yeast, of a suitable sugar salt-buffer mixture.

The thiamin concentration of the leukocytes and platelets is about 10 times that of the erythrocytes, a distribution which probably reflects the respiratory activity of the white blood cells. The total thiamin of the white cells ranged from 49 to 183 μgm. per 100 ml. and averaged 99.8 μgm. per 100 ml. The total thiamin of the erythrocytes ranged from 3.7 to 38.0 μgm. per 100 ml. and averaged 10.3 μgm.

The concentrations of the pyrimidine accelerator in the white cells ranged from 11 to 50 μgm. per 100 ml. and averaged 32.5 μgm. per 100 ml. The pyrimidine accelerator in these instances accounted for from 16.8 to 64% of the total white cell thiamin measured. In the erythrocytes the pyrimidine accelerator concentrations ranged from 1.3 to 5.0 μgm. and averaged 3.0 μgm. per 100 ml. The pyrimidine accelerator accounted for from 14 to 30% of the total red cell thiamin.

The white blood cell levels of thiamin reflect the thiamin deficiency and saturation of the body. White cells of different individuals do not differ in their capacity to absorb thiamin, but can absorb only a limited, maximum amount of the vitamin.

In the course of its metabolic activity, thiamin probably is broken down to the pyrimidine accelerator, for the intravenous administration of thiamin is followed consistently by significantly increased concentrations of the pyrimidine accelerator in the white cells and in the urine.—J. L. M.

ABELS, J. C., GORHAM, A. T., CRAVER, L., and RHOADS, C. P. [Memorial Hosp., New York, N. Y.] THE MEASUREMENT AND METABOLISM OF THIAMIN AND OF A PYRIMIDINE STIMULATING YEAST FERMENTATION FOUND IN THE BLOOD CELLS AND URINE OF PATIENTS WITH LEUKEMIA. J. Clin. Investigation, 21:177-189. 1942.

The total thiamin levels in the leukocytes and platelets of 33 patients with leukemia ranged from 85 to 600 μgm. per 100 ml. of cells. The average value was 277 μgm. per 100 ml. or about 3 times the normal average. Of the 33 patients 27, or 82%, had white cell total thiamin levels above the highest normal. The levels of the pyrimidine accelerator ranged from 4 to 36 μgm. and averaged 21 μgm. per 100 ml. Whereas the pyrimidine accelerator constituted from 16.8 to 64.0% of the total thiamin of normal white cells, in the leukemic white cells that substance represents only from 2.0 to 16.5% of the total thiamin.

The total thiamin levels in the erythrocytes of leukemic patients varied from 6 to 69 μgm. and averaged 22 μgm. per 100 ml., or about twice the normal average level. Of the patients examined, 35% had levels above the highest normal range. In the erythrocytes, however, the concentrations of pyrimidine accelerator form a normal percentage of the total thiamin (8 to 51%).

No correlation was found to exist between the concentration of blood cell total thiamin and the form, severity, or degree of associated leukemia, nor between the concentration and the sex or age of the patient. Patients with leukemia excrete normal amounts of both thiamin and pyrimidine accelerator in the urine.

The probable explanation for the high concentrations of white cell total thiamin is thought to be an impaired utilization of the thiamin, and not an increased ingestion or faulty excretion of the vitamin, nor the apparent youth of the cells involved. Elevated blood cell concentrations of total thiamin have been found in patients with diseases other than leukemia, namely with Hodgkin's disease and cancer of the gastrointestinal tract, but not in patients with portal hepatic cirrhosis.—J. L. M.

STOUT, A. P. [Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.] IS LYMPHOSARCOMA CURABLE? J. A. M. A., 118:968-970. 1942.

A study of 218 cases of lymphosarcoma with microscopic confirmation of the diagnosis, of which 156 received

some sort of curative treatment. Ninety were seen from 1915 to 1930, of whom 48 were treated and 42 were not. There was a survival rate at 10 years of 14.6% for treated and 2.4% for untreated cases. The 5 year survival rate is 21.8% for treated and 3.2% for untreated cases. Of the patients with lymphosarcoma, 6 died of leukemia. Six patients were symptom-free after 10 years. The best prognosis is given by those patients who have but 1 or 2 localized foci when first brought under treatment.—H. G. W.

Adrenal

CAHILL, G. F., MELICOW, M. M., and DARBY, H. H. [Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.] ADRENAL CORTICAL TUMORS. THE TYPES OF NONHORMONAL AND HORMONAL TUMORS. Surg., Gynec. & Obst., 74:281-305. 1942.

Adrenal cortical tumors may occur with or without hormonal syndromes. The hormonal syndromes may be caused by an excess of androgens, estrogens, or other hormones as yet unidentified. The syndromes are determined by the type and amount of hormone produced and by the sex and age of the patient. The status of the adrenals may be adequately shown by air insufflation x-ray films, and removal of adrenal tumors by the transperitoneal route is best. Acute adrenal deficiency occurs only, or mostly, in those cases showing Cushing's syndrome. The more the syndrome approached the Cushing type, the more pronounced was the amount of cell vacuoles present. The therapy of this acute deficiency is similar to that used in the crisis of Addison's disease. Histologically the tumors that produce the hormonal syndromes have cytoplastic lipoid vacuoles in amounts comparable to the symptoms. It was not possible to decide whether the finding of fuchsinophile granules had any particular association with the various types of cases. Fuchsinophile staining of the cytoplasm was present in all tumors, apparently more pronounced in cases with hormonal syndromes.—H. G. W.

GREENBERGER, M. E., and WINER, J. H. [New York, N. Y.] ADRENAL CORTEX CARCINOMA. PREVENTION OF POSTOPERATIVE ADRENAL INSUFFICIENCY. New York State J. Med., 41:1665-1667. 1941.

Since atrophy of the uninvolved gland occurs in the presence of a tumor of the adrenal cortex, a danger following radical extirpation of a cortical neoplasm is adrenal insufficiency. In the case recorded this danger was avoided by postoperative administration of adrenal cortical hormone and by electrolyte therapy.—M. J. E.

Pancreas

LUKE, J. C. [Royal Victoria Hosp., Montreal, Canada] SUCCESSFUL REMOVAL OF A CARCINOMA IN THE HEAD OF THE PANCREAS. Canad. M. A. J., 45:59-61. 1941.

A case report with details of surgical technic.—M. J. E.

MAGNER, W. [Toronto, Canada] HYPERINSULINISM (A REPORT OF TWO CASES). Canad. M. A. J., 45:49-52. 1941.

One patient was cured of the symptoms of hyperinsulinism by resection of an islet cell adenoma of the pancreas, the second after extirpation of a considerable portion of the pancreas, which contained hyperplastic islet tissue.—M. J. E.

Thyroid

LAHEY, F. H. [Lahey Clin., Boston, Mass.] CARCINOMA OF THE THYROID. Am. J. Roentgenol., 46:469-473. 1941.

Most cancers of the thyroid originate in adenomas which remain benign for some time and subsequently become malignant. Only a small percentage of adenomas become malignant but surgical removal carries a negligible risk and is strongly urged in all cases. There are no satisfactory clinical signs of malignant degeneration in an adenoma. Among the suggestive signs are: change in consistency, loss of sharp outline, fixation, and voice changes. Cancer arising in lateral aberrent thyroid tissue constitutes a special problem. Tumors in this position may easily be misdiagnosed as metastases and an operable tumor given up as hopeless.

Most cancers of the thyroid are now treated by combined surgical and radiation therapy. The tributary veins and a portion of the internal jugular should be removed with the entire involved lobe of the thyroid. Since lymph node metastasis takes place early and wide block dissection is impossible in the neck, postoperative irradiation is indicated.—C. E. D.

WARREN, S. [New England Deaconess, New England Baptist, and Huntington Hosps., Boston, Mass.] THE CLASSIFICATION OF TUMORS OF THE THYROID. Am. J. Roentgenol., 46:447-450. 1941.

The morphological features of thyroid tumors may be correlated fairly well with clinical behavior. The following is an abbreviated form of the classification proposed:—

Benign:

1. Adenoma.
2. Papillary cystadenoma.

Malignant:

Group I. Low or potential malignancy.

1. Adenoma with blood vessel invasion.
2. Papillary cystadenoma with blood vessel invasion.

Group II. Moderate malignancy.

1. Papillary adenocarcinoma.
2. Alveolar adenocarcinoma.
3. Hürthle cell adenocarcinoma.

Group III. High malignancy.

1. Small cell carcinoma (carcinoma simplex).
2. Giant cell carcinoma.
3. Epidermoid carcinoma.
4. Fibrosarcoma.
5. Lymphoma.

—C. E. D.

CANCER CONTROL AND PUBLIC HEALTH

SWAN, J. M. [Rochester, N. Y.] POPULAR EDUCATION AS A FACTOR IN THE SOLUTION OF THE CANCER PROBLEM. New York State J. Med., 41:1849-1853. 1941.

The necessity for lay education in achieving a more universal appreciation of the urgency for early treatment of cancer is discussed.—M. J. E.

Book Reviews

SURVEY OF COMPOUNDS WHICH HAVE BEEN TESTED FOR CARCINOGENIC ACTIVITY. By Jonathan L. Hartwell, Research Fellow, National Cancer Institute, National Institute of Health, United States Public Health Service. Federal Security Agency, Washington, D. C. 1941. 371 pages (multilithed). Available for free distribution.

Here, collected for the first time in one volume, are all the pertinent data dealing with 696 chemical compounds tested in animals for carcinogenic potency up to the beginning of the year 1940. From the various papers reviewed the important facts have been abstracted with the purpose of obtaining a comprehensive survey of a single limited field—that of chemical carcinogenesis. Included are some material from the National Cancer Institute as yet unpublished, and much in obscure foreign papers that has been virtually inaccessible hitherto. This latter the author has consulted either in the original or in translation.

The volume is arranged in three parts. An introduction together with a note on the scope of the work and on chemical considerations covers 11 pages. Under the chapter on chemical considerations the author discusses the nomenclature of the compounds commonly employed in the ever enlarging field of experimental cancer research. This will be of particular value to new investigators. The main body of the work is arranged in tabular form, covering 284 pages in which the material is grouped according to chemical compound. Under each compound the pertinent data are listed in 10 categories which include the reference, the animal tested together with its sex and strain, the preparation and dose of the compound, site and route of administration, number of animals with induced tumors, period of survival of the animal, duration of the experiment, and remarks.

Only single chemical compounds are considered, such complex mixtures as the tars derived from a variety of materials, irradiated sterols, and articles of diet being excluded, as are mixtures of two or more pure compounds given simultaneously. Generally included, however, are crude grades of definite chemical individuals. The guiding principle has been to consider only such mixtures as render it feasible to determine to which constituent the given effects are attributed. Both inorganic and organic compounds are treated, and radioactive elements are also represented, since although their action may be generally due to physical radiation in many cases the element is applied directly to the tissue and the possibility of chemical action cannot be rejected.

The author emphasizes the incompleteness of the data on most of the compounds listed, with the consequent limitation that must be imposed on deductions drawn from them, and enters a plea for the publication of more complete data in the future, doubly necessary in long-term work in which repetition by other laboratories is so costly. The most obvious needs are for more complete investigation of compounds already studied in a preliminary fashion and for filling large gaps in the list of new substances which it would seem desirable to test. As an example, the field of the biologically occurring steroids is cited as hardly having been opened, although experiments of long duration have shown that certain members of this group, notably the estrogens, may induce tumors locally at the site of injection as well as in distant organs. Considering that these compounds occur in nature, an inquiry into the structural features responsible for their carcinogenic properties would appear to offer considerable promise of a greater understanding of the etiology of some types of cancer in man.

The author points out that in the United States there is an increasing amount of research being carried out on the numerous general problems depending upon chemical carcinogenesis, particularly the correlation of carcinogenic activity with chemical structure. It is impressive that the 192 compounds which have been proved actively carcinogenic are found in a great variety of chemical classes, and that substances of widely differing chemical structure possess similar carcinogenic activity; whereas, on the other hand, of two given chemical substances of the closest structural similarity one may, and the other may not, be active. These considerations may at first thought make it seem hopeless to connect carcinogenicity in any way with chemical structure. However, it is the author's opinion that there is no justification for relaxing efforts in this direction of approach. Indications of what points should be revealed by studies along this line are the already growing ideas of the organ and tissue specificity and species selectivity among certain carcinogenic compounds. Further careful studies of compounds previously inadequately tested and of compounds not yet employed, may reasonably be expected to throw light on the etiology of different types of cancer.

The monograph is rendered easily usable by the inclusion of four indexes, which cover the route and site of application, species of animal tested, site of tumor development, and vehicle in which the carcinogenic agent is carried. Finally, there are also an index of compounds and a bibliography.

HAROLD L. STEWART

A STUDY OF THE BLOOD IN CANCER, WITH SPECIAL REFERENCE TO THE NEEDS OF THE TUMOUR CLINIC. By O. Cameron Gruner, M.D. Lond. Renouf Publishing Co., Montreal, Canada. 1942. XI + 100 pages; 40 illustrations and 32 tables. Price $4.00.

This monograph describes in detail a microscopic analysis of the formed elements of the blood which, as a diagnostic test for malignant disease, is said to be reliable in from 83 to 97 per cent, according to the type of case.

Though the author acknowledges that the individual features which he notes in these cells are found in various other morbid, and even in physiological, states, the simultaneous occurrence of several of them has been seen rarely, if ever, in any disease but cancer.

At one end of the scale there is a combination which is so significant as to permit an unqualified diagnosis of

cancer, while at the other are findings which are entirely incompatible with the presence of this disorder. Between these two extremes there lies a large number of cases where the readings cannot be so definite and where it can be said only that the chances of cancer are such-and-such a percentage.

Finally, as with all laboratory tests, there are false negatives and false positives. These the author regards as a challenge to further investigation, for if it could be learned why these cases were missed an important clue to the nature of cancer might be discovered.

In making a diagnosis of malignant disease the author relies upon such characteristics of the blood as increased viscidity, absence of rouleaux in fresh preparations, tight cohesion of erythrocytes into masses with sharp contours, a tendency of the erythrocytes in the centers of these masses to undergo hemolysis, early appearance of a dense fibrin network, notable absolute and relative decrease in the number of small lymphocytes, neutrophile nuclei of highly irregular shape, polymorphous and very large platelets, abnormal contours of the monocyte nuclei, and the occurrence of mitochondrial inclusions in the mononuclear leucocytes. These are given approximately in order of importance but the last is said to clinch the diagnosis, turning the scale in favor of malignant disease.

The book, which is generously illustrated with graphs, half-tones, and color plates, closes with a description of the 20 tests, out of some 250 in the literature, which the author regards as most useful.

WILLIAM H. WOGLOM

A SYMPOSIUM ON RESPIRATORY ENZYMES. The University of Wisconsin Press, 811 State Street, Madison, Wisconsin. 1942. 281 pages. Price $3.00.

Because of the interrelationship of vitamins and respiratory enzymes in cellular metabolism, a discussion by the enzyme chemists and nutritionists on the "Respiratory Enzymes and the Biological Action of the Vitamins" was sponsored jointly by the Universities of Wisconsin and Chicago.

This book includes the lectures and discussions on respiratory enzymes held at the University of Wisconsin, September 11-17, 1941. It contains the following lectures: Intermediate Carbohydrate Metabolism by O. Meyerhof; Oxidative Mechanisms in Animal Tissues by E. G. Ball; Pasteur Effect by F. Lipmann; Oxidases, Peroxidases, and Catalase by K. G. Stern; Nicotinamide Nucleotide Enzymes by F. Schlenk; The Flavoproteins by T. R. Hogness; Cytochromes by E. Stotz; Phosphorylation of Carbohydrates by C. F. Cori; Metabolic Cycles and Decarboxylation by E. A. Evans, Jr.; and Transamination by P. P. Cohen. The following Discussions also are included: Hydrogen Transport by K. A. C. Elliot, E. G. Ball, F. Lipmann, K. G. Stern, E. Hass, and E. Stotz; Phosphorylation by H. M. Kalckar, O. Meyerhof, M. J. Johnson, and F. Lipmann; Bacterial Respiration by H. G. Wood, R. H. Burris, C. H. Werkman, E. S. Guzman Barron, P. W. Wilson, and F. F. Nord; and Animal Tissue Respiration by E. Shorr, K. A. C. Elliot, V. R. Potter, A. E. Axelrod, F. Bernheim, E. S. Guzman Barron, and F. J. Stare.

The volume contains also a Discussion on Tumor Respiration, which includes the following topics: Characteristics of Tumor Respiration by K. A. C. Elliot; Phosphorylation Theories and Tumor Metabolism by V. R. Potter; On the Specificity of Glycolysis in Malignant Liver Tumors as Compared with Homologous Adult or Growing Liver Tissues by D. Burk; and The Effects of Certain Diamines on Enzymes Systems, Correlated with the Carcinogenicity of the Parent Azo Dyes, by C. J. Kensler.

It may be mentioned in passing that the book also includes candid photographs of many of the participants in the symposium.

Though all this is an exhaustive list of topics, which of necessity must be comparatively limited in development, those chosen are for the most part very adequately presented by their respective authors. Beginners in this field will probably have little difficulty in extracting the information and understanding the interpretations and theories contained in the book, for most of the chapters are written with the simplicity only attained by investigators who are well acquainted with and understand their material. Not only does the book include the latest developments and interpretations of many findings, but it also serves the useful function of pointing out the many problems that await solution before one can more fully understand normal cellular metabolism and perhaps subsequently tumor metabolism. After finishing a book of this sort one finds himself agreeing with Otto Meyerhof, who points out that "our pride in the progress achieved in the last decades must be tempered by confession of ignorance regarding many crucial points."

DAVID SHEMIN